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BIOLOGICALLY-BASED KINETIC MODELS FOR CHEMICAL KINETICS IN THE ISOLATED PERFUSED RAT LIVER SYSTEM: BBKM-IPRLR
(BASIC RECIRCULATION MODEL FOR WATER SOLUBLE / LIPID INSOLUBLE CHEMICALS (LOG $P_{OW}<0$))

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

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FOR THE DIRECTOR

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13. ABSTRACT (Maximum 200 words)

The objective of this report is to describe a generic biologically-based kinetic (BBK) model to simulate the kinetics of water soluble - lipid insoluble chemicals (log Po/w < 0) in the isolated perfused rat liver (IPRL) system. Such a model should have sufficient flexibility to allow for the inclusion of various physiological and biochemical processes into the description of the kinetics of chemicals that fall into this physical-chemical category. The following kinetic processes are incorporated into this model: (1) transport across cellular membranes, both at the sinusoidal-hepatocyte interface and the hepatocyte-biliary canalicular interface, (2) protein binding, both in perfusion medium and intracellular compartments of the liver, and (3) metabolism of the chemical in liver. The initial modeling efforts resulted in the development of a robust BBK model, named BBKM-IPRLR, that allows for the investigation of the effects of these kinetic process on the behavior of water soluble chemicals in the IPRL system. The generic model is used to demonstrate the effect of various kinetic processes on the observable state variables. Interactions between various kinetic processes are also demonstrated.

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1.0 MODEL STRUCTURE

1.1 INTRODUCTION

The objective of this report is to describe a generic biologically-based kinetic (BBK) model for water soluble - lipid insoluble chemicals (log P_{ow} < 0) in the isolated perfused rat liver (IPRL) system. Such a model should have sufficient flexibility to allow for the incorporation of various physiological and biochemical processes into the description of the kinetics of chemicals that fall into this category. The following factors are incorporated into this model: (1) transport across cellular membranes, (2) protein binding, both in perfusion medium and intracellular compartments of the liver, (3) metabolism of the chemical in liver, and (4) the possibility of biliary excretion. The initial modeling efforts are focused on the development of a robust BBK model that allows for the investigation of the effects of these process on the behavior of water soluble chemicals in the IPRL system.

1.2 GENERAL STRUCTURE OF MODEL

1.2.1 System structure

A schematic diagram of the IPRL system is given in FIGURE 1. The mechanical features of the liver perfusion apparatus are described in Wyman, et al. (1995). The perfusion medium, a physiologically balanced buffer containing the chemical of interest, is pumped through the liver and the outflow from the liver is returned to a reservoir for recirculation. In general, the liver is cannulated via the portal vein in situ and removed from the rat. The isolated liver is connected into the perfusion apparatus and allowed to adjust to the new perfusion conditions for 60 min. At that time, designated t = 0, the chemical of interest is added to the reservoir and rapidly mixed into the bulk of the perfusion medium. The chemical is then recirculated for a given time, usually 120 min. At appropriate time intervals, samples of perfusion medium are collected and analyzed for the parent chemical and its metabolites, if required. In the liver isolation procedure, the bile duct is cannulated and samples of bile are collected over 30 min intervals. Bile flow rate is evaluated as a measure of liver function and collected bile can be analyzed for the parent compound and metabolites to quantitate biliary excretion kinetics.
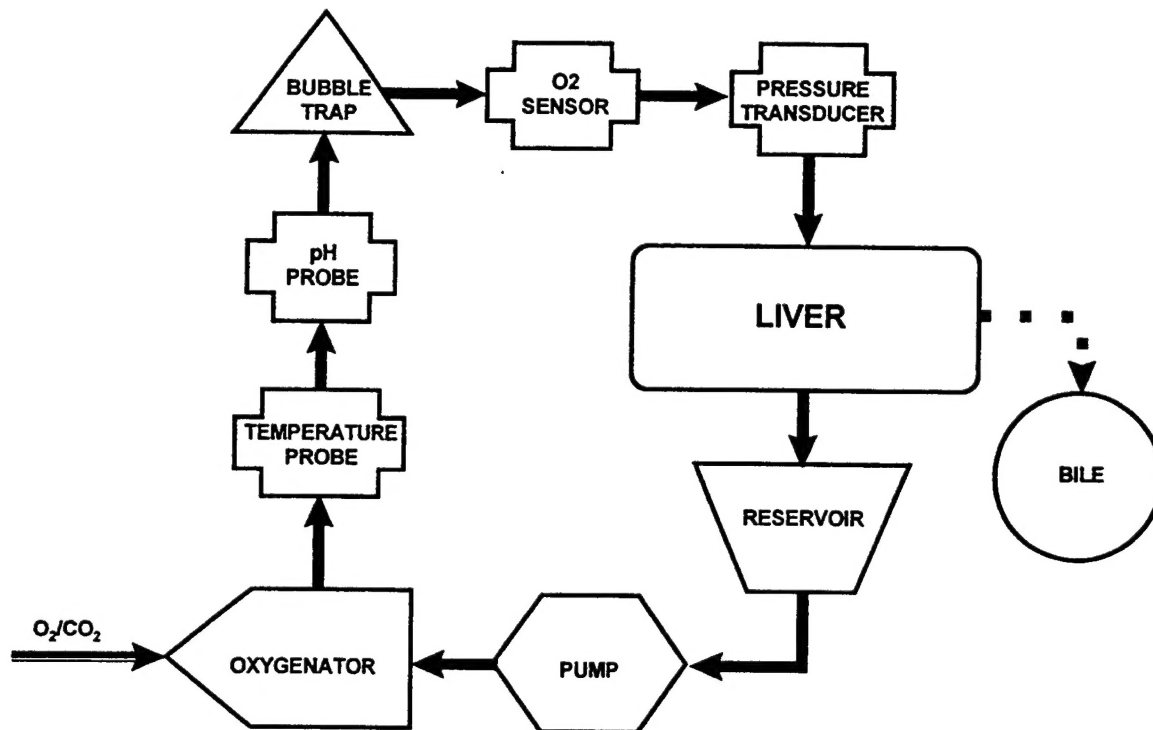
Figure 1: Schematic Diagram of the IPRL System

Perfusion medium is recirculated through a closed-loop system controlled by the pump. The liver is perfused through the portal vein at a flow rate of QL (= 2.4 L/h). Temperature, pH, O_2 saturation and pressure are monitored continuously. Bile is collected via a cannula inserted in the bile duct.

1.2.2 Perfusion conditions

Under standard conditions, the liver is perfused with Krebs-Ringer bicarbonate buffer supplemented with 11.5 mM glucose and 4 % (w/v) albumin. The perfusion medium is oxygenated (95% saturation) and the pH is regulated at 7.4. The temperature of the perfusion medium is maintained at 37°C.

1.2.3 Liver compartmentalization

The compartmental structure of a general organ in a BBK model depends on the degree of complexity required for an adequate description of the kinetic effects to be observed. The possible descriptions range from treating the entire organ as a single compartment, on one hand, to a detailed description of the tissue structure and the use of partial differential equations, on the other. In the intermediate situation, a perfused organ can be resolved into five well-mixed theoretical compartments, as illustrated in FIGURE 2. In this general case, the organ is perfused through the vasculature. Chemical present in the perfusion medium can distribute directly into the interstitial space and further penetrate into the intracellular water space and/or partition into tissue lipid compartments.

Based on this physiological description of a general organ, the liver can be divided into the five physically identifiable compartments:

(1) Vascular space - the aqueous space contained within the tissue vasculature as defined by vascular endothelial cells (arteries, veins and sinusoids).

(2) Interstitial space - the aqueous space lying between the vascular space and the intracellular fluid space, excluding connective tissue. In the liver, the equivalent of the interstitial space is the Space of Disse.

(3) Intracellular space - the cytoplasmic and organelle aqueous space within cells.

(4) Lipid space - cell membranes and intracellular lipid droplets.

(5) Solid phase - connective tissue and mineral phase (there is little solid phase in the liver).

The liver requires special treatment due to the unique structure of the sinusoids. Goreski (1980), using indicator dilution techniques, has demonstrated that albumin rapidly penetrates into a space almost equivalent to the total extracellular space

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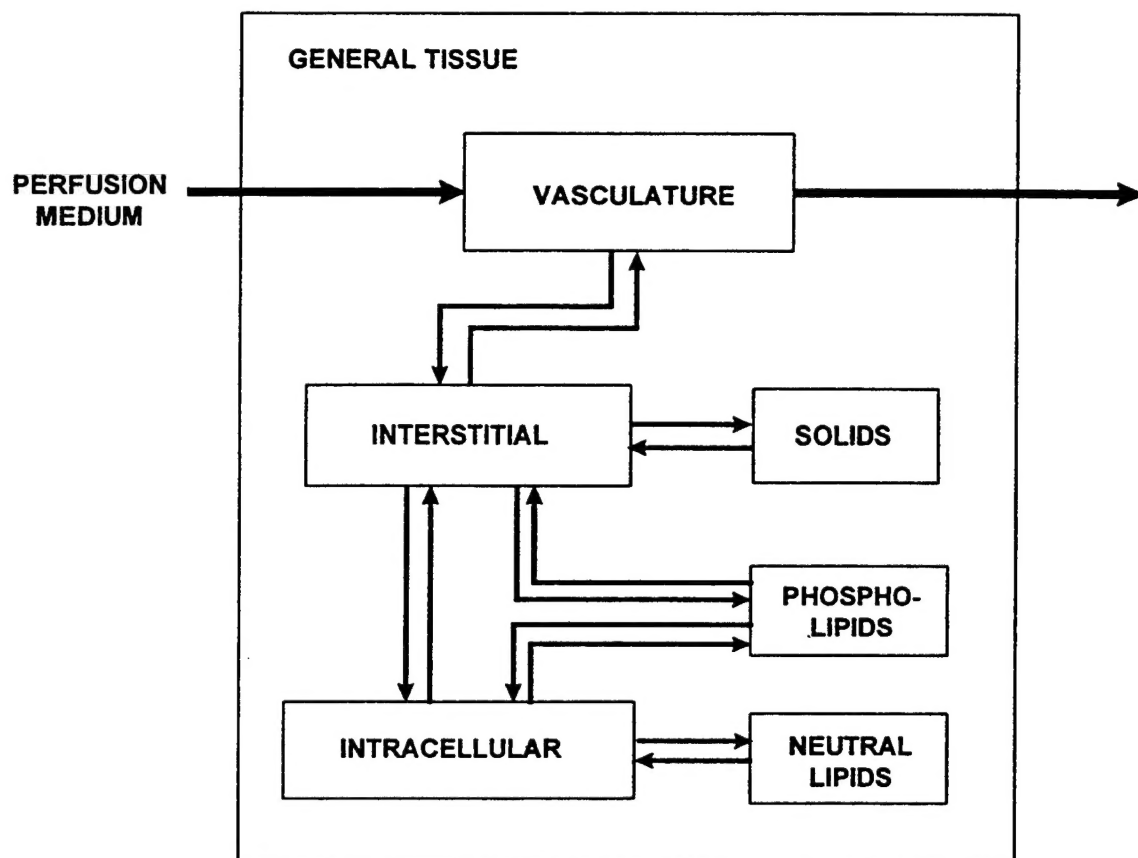
Figure 2: Compartmentalization of a General Perfused Organ.

The general organ can be divided into six theoretical compartments, the vasculature through which the perfusion medium flows, the interstitial water space which connects the vasculature space with the intracellular water space, the intracellular water space, the phospholipid compartment consisting of cellular and organelle membranes, the neutral lipids which represent stored fat in the tissue, and the solid compartment which consists mostly of the connective tissue and basement membranes in an organ. In the general case, a chemical can distribute into any of these compartments in an organ.

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penetrated by sodium. The conclusion is that the vascular and interstitial spaces are in close communication in the liver and thus should be treated as a single compartment. This observation has been incorporated into IPRL model and, therefore, the liver consists of only two aqueous compartments, the sinusoidal space, including the Space of Disse, and the intracellular space. It is further assumed that chemicals that fall into the water soluble - lipid insoluble category do not accumulate in either the lipid space or the solid phase of a tissue, therefore the lipid and solid compartments are not kinetically connected to the other compartments, even though they are retained in the description to account for total organ weight. If a chemical is both water soluble and also has a significant degree of solubility in the lipid phase, then partitioning into lipids would be important and should be included in the kinetic model. These assumptions on the structure of the perfused liver for water soluble - lipid insoluble chemicals are summarized in Figure 3.

For the IPRL model, the liver weight is partitioned into:

$$\text{Sinusoidal space:} \quad V_{LS} = F_{VLS} * W_L$$

(1a)

$$\text{Intracellular space:} \quad V_{LI} = F_{VLI} * W_L$$

(1b)

$$\text{Lipid space:} \quad V_{LF} = F_{VLF} * W_L$$

(1c)

$$\text{Solid space:} \quad V_{LSD} = F_{VLSD} * W_L$$

(1d)

where the V_{Li} {L} are the volumes of the liver compartments, the F_{VLi} {L/Kg} are the weight to volume conversion fractions and W_L {Kg) is the weight of the liver.

1.2.4 Parameters/Parameter estimation

From the point of view of the general model structure, there are six chemical independent model parameters: (1) the volume of the reservoir, V_R, (2) the perfusion rate, Q_P, (3) the weight of the liver, W_L, (4) the fraction of the liver that is sinusoidal space, F_{VLS}, (5) the fraction of the liver that is intracellular space, F_{VLI}, and (6) the conversion factor to compute the surface area of the sinusoidal - intracellular (S-I) interface. The volume of perfusion medium in the reservoir is controlled by the experimenter. In most experiments, the default value is 0.200 L. The perfusion rate is also controlled by the experimenter and is set high enough to
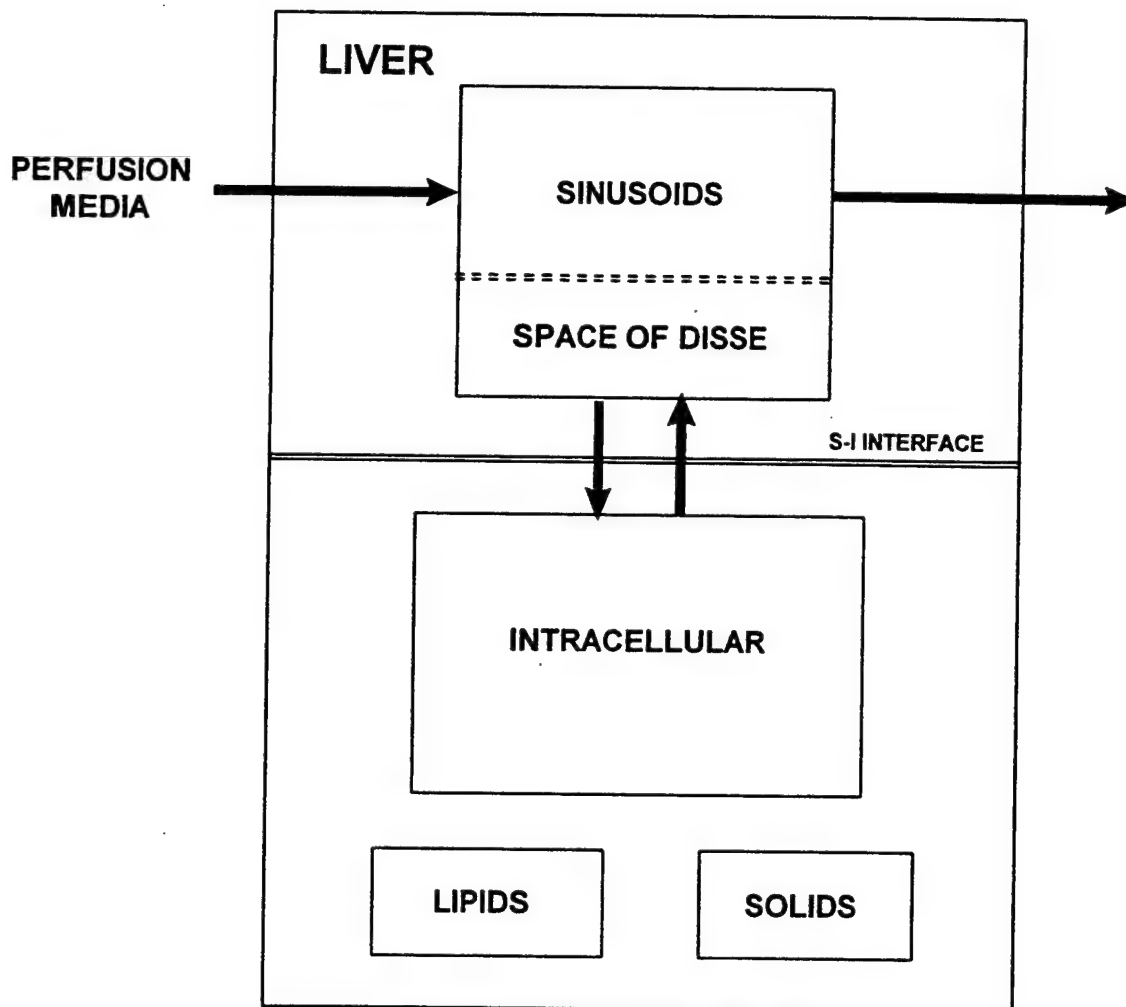
Figure 3: The Organ Compartments for the IPRL.

For the IPRL, the vascular and interstitial spaces are combined to form the extracellular water space, referred to as the sinusoidal space, and the lipid and solid spaces are present but are not connected to the system due to the assumptions about the nature of the chemicals under investigation. These assumptions must be reevaluated for any particular chemical.

assure full oxygenation of the liver. The default value is 2.4 L/h (40 ml/min). The liver weight is determined by the size of the liver donor. For most experiments, the rats (usually Fisher 344 rats) weigh between 0.250 and 0.300 Kg, thus, the livers are in the range of 0.007-0.009 Kg. The fraction of the liver that is considered the sinusoids is about 0.21 (or 21% of the liver weight is associated with water in the sinusoids). This fraction is not well determined, but if a radio-labeled chemical is used in the study, this fraction can be estimated in individual experiments by perfusing the liver sinusoids at the end of an experiment with chemical-free media and determining the total radioisotope in the sinusoids {CPM}. Dividing by the activity in the perfusion medium {CPM/L} will give the volume of the sinusoids and dividing by the liver weight will give F_{VLS}. The volume of the intracellular space can be estimated by determining the total water content of the liver {L/Kg} from the wet weight/dry weight ratio. Subtracting the sinusoidal water space from the total water space will give the intracellular water space, from which the fractional intracellular water space can be calculated. The last structural parameter is the conversion factor that is used to determine the area of the S-I interface. This parameter cannot be measured directly at the present time (see discussion in the next section).

1.3 MEMBRANE TRANSPORT

1.3.1 Conceptual description

Transport across membrane barriers in the liver is chemical specific and can occur by several mechanisms, including simple diffusion, facilitated diffusion, active transport or vesicle mediated transport (pinocytosis or receptor mediated endocytosis). This spectrum of transport mechanisms range from linear, energy independent processes to complex non-linear, energy-dependent processes. Detailed knowledge of the transport mechanisms is essential for an accurate description of kinetics over a broad concentration range. However, the use of a linear and a hyperbolic (Mechaelis-Menten) term can empirically describe a wide range of kinetic behaviors.

1.3.2 Mathematical description

The mass transport rate parameters, CT_{LSI} and CT_{LIS} {L/h}, are used in the IPRL model to describe the movement of free chemical across the barrier separating the sinusoidal and the intracellular compartments of the liver. These terms are defined as

$$CT_{Lij} = \frac{Rate\ of\ Unidirectional\ Mass\ Transport\ (j \to i)}{Available\ Concentration\ in\ Compartment\ j} \tag{2}$$

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Thus, the product of the mass transport rate parameter and the available concentration of the chemical in the compartment from which the mass flow originates gives the unidirectional flux of the chemical. The mass transport parameters can be concentration dependent for non-linear transport processes.

In the IPRL model, it is assumed that both simple diffusion and carrier mediated transport can be involved. For the simple diffusion component it is assumed that the associated transport rate parameters are symmetrical, i.e., given equal concentration gradients across the membrane barrier in the forward or reverse directions, the rate of chemical transport by simple diffusion will be equal in either direction. Therefore, the simple diffusion component of the mass transport rate parameter is of the form

$$CT_{LSI}(\text{diffusion } I{\rightarrow}S) = CT_{LIS}(\text{diffusion } S{\rightarrow}I) = 3.6*PT_{LSI}*A_{LSI} \qquad (3)$$

where PT_{LSI} {cm/sec} is the diffusional permeability of the S-I interface barrier for free chemical and A_{LSI} {cm^2} is the surface area of the S-I interface in the liver. The factor 3.6 is included to convert from units of cm^3/sec to L/h.

The mediated transport contribution to the transport rate parameter for chemical flux at the S-I interface of the liver is assumed to be of the saturable hyperbolic form:

$$CT_{LSI}(\text{mediated transport } I{\rightarrow}S) = 3.6*T_{LSI}*A_{LSI}/(X_{LI} + KT_{LSI}) \qquad (4)$$

where T_{LSI} {nmoles/sec*cm^2} is the maximum rate of transport of the chemical by the carrier mediated system at the S-I interface of the liver tissue, A_{LSI} {cm^2} is the surface area of the S-I interface in the liver, X_{LI} {μM} is the free chemical concentration in the intracellular compartment of the liver and KT_{LSI} {μM} is the apparent dissociation constant for the chemical - carrier complex. The factor 3.6 is included in this equation to convert from nmoles/sec to μmoles/h.

Transport across the I-S interface by the carrier mediated process may or may not be symmetrical. To take into consideration the assymetrical possibility, the mediated transport component of the reverse transport parameter, CT_{LIS}, is

$$CT_{LIS}(\text{mediated transport } S{\rightarrow}I) = 3.6*T_{LIS}*A_{LSI}/(X_{LS} + KT_{LIS}) \qquad (5)$$

where T_{LIS} and KT_{LIS} do not necessarily equal their counter-parts for the reverse I→S transport process. At the S-I interface of the liver there are a total of four model parameters to describe mediated transport (T_{LSI}, KT_{LSI}, T_{LIS}, and KT_{LIS}). If the

mediated transport process is symmetrical, the number of parameters reduces to two. Note, the surface area parameter, A_{LSI}, is the same for mediated transport as for the simple diffusion component. As in the case for diffusion, the individual parameters T_{Lij}, and A_{LSI} cannot be determined independently at the present time, only their product is estimated by fitting kinetic data. Finally, the saturable hyperbolic expression used to describe carrier mediated transport is an approximation that applies only when there are no interactions between forward and reverse transport. More complex representations for carrier mediated transport must be included in the model to account for these interactions.

1.3.3 Parameters/Parameter Estimation

A key model parameter in this description is the S-I interface surface area, A_{LSI}. A_{LSI} is calculated using the physiological scaling parameter, γ_{LSI} {cm^2/Kg}, which relates the surface area at the S-I interface to the liver weight.

$$A_{LSI} = \gamma_{LSI} * W_L \tag{6}$$

At the present time, experimental techniques are not available to independently evaluate either PT_{LSI} or γ_{LSI}, thus by default the permeability*area product must be treated as a combined parameter. In the future, it is planned to develop new experimental techniques to obtain independent measurements of membrane permeabilities and surface areas. When such techniques are developed, the experimentally determined values for PT_{LSI} and γ_{LSI} can be incorporated directly into the model. In the meantime, the modeling strategy is to set $\gamma_{LSI} = 1.0$ as the default and allow the value of PT_{LSI} to represent the permeability*area product {cm^3/sec}.

In the long term, the development of experimental techniques to evaluate membrane transport mechanisms and quantitate kinetic parameters rapidly are needed.

1.4 PROTEIN BINDING

1.4.1 Conceptual description

Many chemicals are strongly, yet reversibly, bound to proteins. In plasma, the major binding protein is albumin, although other proteins are known to be involved in particular cases. Bovine serum albumin is present in the perfusion medium used in the IPRL studies. In the intracellular spaces of the liver, high molecular weight macromolecules are the major binding ligands. The IPRL model allows for the description of these reversible binding processes. If irreversible

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binding occurs, such as adduct formation between reactive metabolites and cellular macromolecules, a different approach must be used.

Since most protein binding reactions occur rapidly, it is assumed that the free and protein bound chemical in any given compartment is in quasi-equilibrium, i.e., even though the concentrations are changing in time, at any given instant the distribution between free and bound species are close enough to equilibrium that the error associated with making this assumption is negligible (i.e., binding kinetics are fast relative to distribution kinetics). Thus, during the simulation process, the model program first computes the change in the total mass of chemical in a given compartment and then computes the distribution of the chemical among all possible species assuming an equilibrium condition exists. Having computed this distribution, the equilibrium concentrations of the various species of the chemical at this time step are used as initial conditions for the next time step in the simulation.

1.4.2 Mathematical description

The distribution of the chemical among free and bound species in a compartment is computed by solving a set of simultaneous, non-linear algebraic equations and is executed in the model by a FORTRAN subroutine called DIST11. This subroutine is capable of determining the distribution of a chemical between the free and bound form, assuming the presence of one binding ligand. If more complicated situations arise DIST22 can be used. DIST22 can determine the distribution of two chemicals competing for binding to up to two ligands. DIST22 evaluates how many chemicals (up to two) and how many ligands (up to two) are present in a compartment before selecting the optimum solution procedure. If there is only one chemical present, the distribution is computed by an algebraic relationship. If two chemicals are competing for binding, the solution is obtained by a Newton-Ralphson algorithm for solving simultaneous nonlinear equations.

As an example, consider the computation of the distribution of a single chemical (T) between the free and bound forms in the sinusoidal compartment of the liver in the presence of one binding ligand, e.g., bovine serum albumin. The total amount of the chemical in the compartment, M_{LS} {μmoles}, is determined by the solutions of the mass transport differential equations (see below). Calling DIST11 with M_{LS} as an argument, the free and bound concentrations of the chemical in the sinusoids are computed. Several parameters are required by DIST11 to solve for the free and bound concentrations and are passed to the subroutine as arguments in the CALL statement. In this particular case, the required parameters are: (1) UB_{LS} {μM}, the binding capacity of the ligand for the chemical in the sinusoids ($UB_{LS} = N *$ concentration of ligand in perfusion medium, where N is the number of identical binding sites on the ligand), (2) KB_{LS} {μM}, the dissociation constant for the chemical from the ligand, and (3) V_{LS}, the volume of

the sinusoidal space in the liver. In this case, the free and bound concentrations are given by

$$X_{LS} = \frac{1}{2}\left(\sqrt{(KB_{LS} + UB_{LS} - Z_{LS})^2 + 4 * KB_{LS} * Z_{LS}} - (KB_{LS} + UB_{LS} - Z_{LS})\right) \qquad (7a)$$

$$Y_{LS} = \frac{UB_{LS} * X_{LS}}{X_{LS} + KB_{LS}} \qquad (7b)$$

where X_{LS} {μM} is the free concentration of the chemical in the liver sinusoids, Y_{LS} {μM} is the bound concentration of the chemical and Z_{LS} {μM} = M_{LS}/V_{LS} is the total concentration of the chemical in the liver sinusoidal compartment.

The binding parameters - binding capacity and dissociation constant - are chemical specific parameters and must be determined for each case.

1.4.3 Parameters/Parameter Estimation

The parameters required for estimating the distribution of a chemical between the free and bound species are the binding capacity and the binding affinity. Values for these parameters must be determined for both the parent chemical and its metabolites. Experimentally, these parameters can be estimated from *in vitro* binding studies using dialysis or ultrafiltration techniques. Binding parameters are chemical specific parameters and must be determined on a case-by-case basis. Equilibrium dissociation constants are used in the model.

1.5 METABOLISM

1.5.1 Conceptual description

Many chemicals are subject to enzymatic modification in biological systems. The liver is often considered the main site of such metabolism. In order to describe metabolism, the IPRL model incorporates a metabolic activity term into the differential equation describing chemical kinetics in the liver intracellular compartment. Several assumptions are incorporated into the metabolic component of the liver. First, it is assumed that there is only one metabolic pathway catalyzed by a single enzyme. Second, it is assumed that the enzyme responsible for metabolic activity is located in the intracellular space and derives its substrate from the intracellular aqueous space. Thus, the appropriate substrate concentration

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used in the metabolism term is the free concentration of the chemical in the intracellular space. Third, the enzymatic process obeys the conditions that are necessary for the rate of metabolism to be adequately described by the Michaelis-Menten relationship. Under these assumption, the mathematical form of the metabolism term is defined.

In reality, there may be multiple metabolic pathways, the nature of the enzymatic process may not allow for the Michaelis-Menten relationship, there may be isoforms of the enzyme, there may be limiting cofactors. Any of these factors can alter the correct mathematical description of the enzymatic process. When the model is applied to a particular chemical these issues must be taken into consideration.

1.5.2 Mathematical description

A metabolism term is included in the mass balance differential equation for both the parent and metabolite in the intracellular compartment of the liver. The rate of formation of the metabolite (M) is equal to:

$$R_{METABOLISM} = SV_{MAX}*W_L*X_{LI}/(X_{LI} + KM_{LI}) \tag{8}$$

where $R_{METABOLISM}$ {μmoles/h} is the total rate of formation of the metabolite in the liver (= the rate of metabolism of the parent chemical), SV_{MAX} {μmoles/h*Kg} is the maximum rate of enzymatic metabolism of the chemical in the liver per Kg of liver weight, W_L {Kg} is the weight of the liver, X_{LI} {μM} is the free concentration of the parent chemical in the hepatic intracellular space, and KM_{LI} is the K_M {μM} for the enzyme using the parent chemical as substrate.

1.5.3 Parameters/Parameter estimation

The determination of metabolic parameters from *in vitro* methods has been a topic of discussion for many years. Much of the discourse has focused on how to extrapolate *in vitro* measurements to the *in vivo* situation. A portion of the discrepancies that have been noted in the past can be attributed to not adequately relating what is measured *in vitro* to what is actually required for the *in vivo* model. K_Ms measured *in vitro* are closely related to free concentrations of the chemical at the site of metabolism and can be significantly different from the "blood concentration". However, if care is taken to relate the parameters measured *in vitro* to the appropriate model parameters, the correlation is usually better. Metabolic parameters can be measured in microsomal/cytosolic preparations and isolated hepatocytes.

1.6 BILIARY EXCRETION

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1.6.1 Conceptual description

Biliary excretion of the parent chemical and/or metabolites is an important kinetic pathway for many chemicals. Full knowledge of the mechanisms of biliary excretion for specific chemicals is often not available. Several distinct pathways are possible. Pericellular pathways imply that chemicals can reach the bile canaliculi by penetration of the tight junctions between hepatocytes that define the canaliculi. Transcellular pathways involve transport of the chemical across two cellular membranes, one at the sinusoidal side of the hepatocyte and the other at the canalicular side. Specific transport processes can be active at each location that can result in unidirectional transport of a chemical or its metabolites into the bile. In addition, metabolism of the parent chemical, such as conjugation with glutathione, can result in metabolites that are preferentially transported into bile. New experimental techniques are needed to better define the biliary transport mechanisms that occur in the liver and provide quantitative measures of the kinetic parameters.

In general, when modeling the elimination of a chemical or its metabolites in the bile, the cumulative excretion is computed, i.e., the total mass eliminated up to a given time. The modeling of the concentration of the chemical in the bile is a more difficult task. The concentration in the bile is determined by the rate of mass transport from the hepatocytes into the bile and the rate of bile formation. If it is assumed that the rate of bile formation {L/h} is constant, then the concentration can be calculated as the ratio of the rate of mass transport to the rate of bile formation. However, many chemicals will affect the rate of bile formation. In this case, the effect of the chemical on bile formation must be taken into account empirically since the mechanistic basis of the effects of chemicals on bile formation are poorly understood for most chemicals. Taking these confounding issues into consideration, the usual approach is to model the cumulative biliary elimination rather than the concentration in the bile.

1.6.2 Mathematical description

In the IPRL model, two transport processes that move the parent chemical, or its metabolites into the bile are defined. The first process is a linear process that results in first order elimination into bile from the free intracellular pool. The second process is saturable and is approximated by a Michaelis-Menten type equation. Thus, the rate of transport of parent chemical into bile is

$$R_{BILE} = SB_1 * W_L * X_{LI} + SB_2 * W_L * X_{LI}/(X_{LI} + KT_B) \qquad (9)$$

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where R_{BILE} {μmoles/h} is the rate of chemical transport into bile, SB_1 {L/h*Kg} is the specific proportionality constant for the linear transport process, W_L {Kg} is the weight of the liver, X_{LI} {μM} is the free concentration of the chemical in the intracellular compartment, SB_2 {μmoles/h*Kg} is the specific maximum rate of mediated transport of the chemical into bile and KT_B {μM} is the free intracellular concentration when the rate of transport is at one-half the maximum rate. Similar expressions for the metabolites can be used.

1.6.3 Parameters/Parameter estimation

Currently, there are few *in vitro* techniques to evaluate biliary excretion parameters. Membrane vesicle studies may prove predictive, but the required techniques are not fully developed and validated. In this situation, the biliary excretion parameters must be empirically determined by experimental studies in isolated liver or *in vivo* studies.

1.7 MASS BALANCE RELATIONSHIPS AND DIFFERENTIAL EQUATIONS

1.7.1 Conceptual description

The differential equations describing the rate of change of the total mass of the chemical in the liver compartments can be written in a general form. We will make the following assumptions:

(1) The liver sinusoidal/intracellular compartments can be treated as well mixed, i.e., this assumption implies that concentration gradients do not exist within liver compartments, in particular as a function of distance from the portal/arterial to central venous sides of the liver.

(2) Accumulation of water soluble chemicals in the lipids and solids compartments of the liver is negligible. Thus, these compartments are not connected with other compartments in the model.

(3) Transport of the chemical between sinusoids and the intracellular compartment within the liver can be the rate limiting process, in such cases the "diffusion limited" condition is invoked. However, if the mass transport rate parameters of the chemical are very large, relative to perfusion rate, the system should behave as a "perfusion limited" model.

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(4) Only free chemical can exchange between the sinusoids and intracellular compartments. If any of the protein bound species can be specifically transported across cellular membranes, then the differential equations must be modified to take these pathways into consideration.

(5) Transport of free chemical between compartments can be a result of both simple diffusion and a mediated transport pathways.

(6) Metabolism of the parent compound is dependent on the concentration of the free chemical in the intracellular water space.

(7) Only the free species of the parent chemical or its metabolite is available for transport into the bile.

Schematic diagrams for the IPRL models for the parent chemical and the metabolite are given in FIGURE 4. The exact differential equations that describe the rate of change of total chemical for each compartment in the IPRL system are given below.

1.7.2 Mathematical description

1.7.2a Parent chemical in the sinusoidal compartment of the liver

The differential equation that describes the rate of change of the total mass of the parent chemical in the sinusoidal compartment of the liver (denoted by the suffix LS) is

$$RMT_{LS} = Q_L*ZT_R + CT_{LSI}*XT_{LI} - CT_{LIS}*XT_{LS} - Q_L*ZT_{LS} \qquad (10)$$

where RMT_{LS} {μmoles/h} is the rate of change of the total mass of the parent chemical T present in the sinusoidal compartment of the liver, Q_L {L/h} is the flow of perfusion medium to the liver, ZT_R {μM} is the total concentration of chemical in the perfusion medium (= concentration in the reservoir), including free and bound species, CT_{LSI} and CT_{LIS} {L/h} are the mass transport rate coefficients for free chemical flux between the sinusoidal and intracellular spaces of the liver, XT_{LS} and XT_{LI} {μM} are the free chemical concentrations in the sinusoidal and intracellular compartments, and ZT_{LS} (μM) is the total concentration of the chemical in the sinusoidal compartment of the liver,

$$ZT_{LS} = MT_{LS}/V_{LS} \qquad (11)$$

A
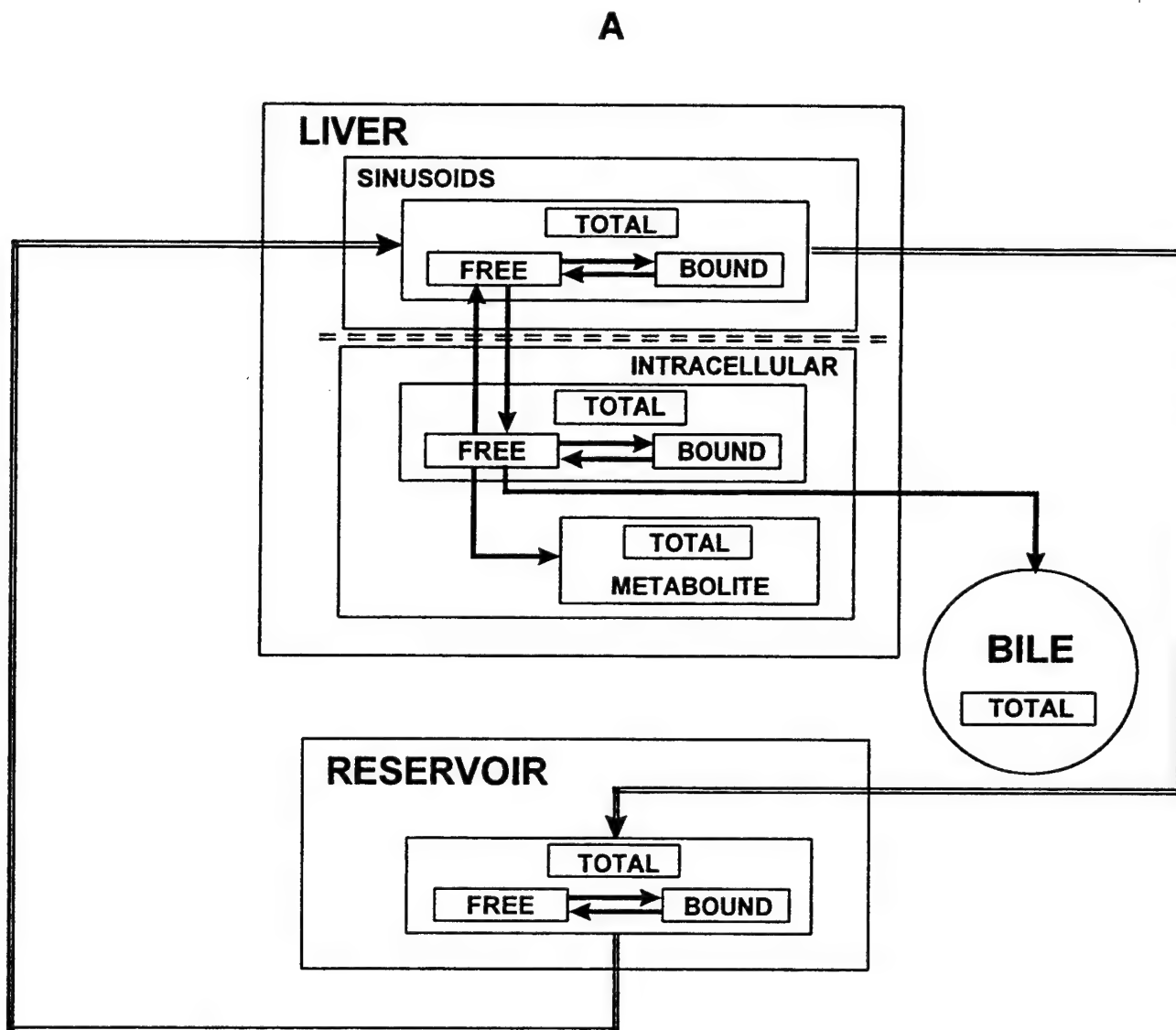
Figure 4: Conceptual Model for the Parent Chemical (A) and the Metabolite (B) in the IPRL System.

Test chemical in the perfusion medium re-circulates through the liver via the reservoir. The total chemical in any compartment is distributed between the free and bound species. Within the liver the parent chemical can be excreted in the bile or converted to the metabolite. The metabolite can either circulate in the system or be excreted in the bile.

16

B

LIVER

SINUSOIDS

TOTAL

FREE ⇄ BOUND

INTRACELLULAR

TOTAL

FREE ⇄ BOUND

FREE

PARENT

BILE

TOTAL

RESERVOIR

TOTAL

FREE ⇄ BOUND
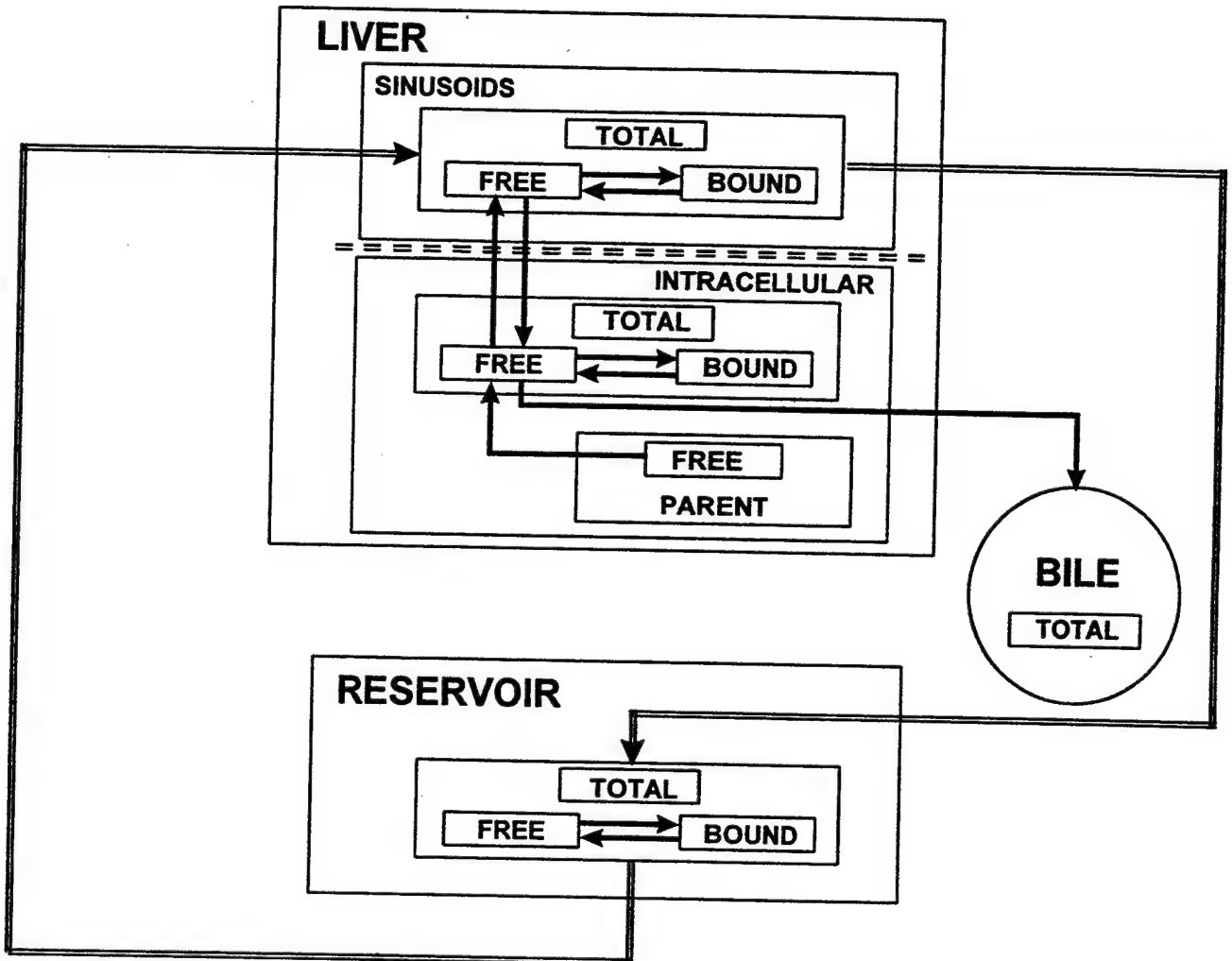
The model uses the mass balance equations to compute the state variable representing the total amount of the chemical in the sinusoidal compartment of the liver, MT_{LS} {μmoles}. Given the total mass of the chemical in the compartment, the distribution of chemical between the free and bound species is determined using DIST11 as described in the section on protein binding.

1.7.2b Parent chemical in the intracellular compartment of the liver (LI)

The rate of change of total parent chemical in the intracellular space of the liver is described by

$$RMT_{LI} = CT_{LIS}*XT_{LS} - CT_{LSI}*XT_{LI} - RT_{METABOLISM} - RT_{BILE} \qquad (12)$$

where the first two terms represent mass transport between the sinusoidal and intracellular spaces (corresponding terms appear in the equation for T in the sinusoidal space), $RT_{METABOLISM}$ is the total rate of metabolism of T and RT_{BILE} is the rate of biliary excretion of the parent chemical.

Again, the same approach is used to compute the distribution of the chemical between the free and intracellular high molecular weight macromolecular (HMW) bound species in the intracellular space of the liver. In the IPRL model, it is assumed that only one class of HMWM ligands are present in the intracellular space, however this assumption can be expanded to take into consideration multiple classes of HMWM ligands. The subroutine DIST11 is called using the appropriate arguments and the free and bound species of the chemical are computed. The parameters required to compute the distribution are: UBT_{LI} {μM}, the binding capacity of HMWM intracellular ligands for the parent chemical, KBT_{LI} {μM}, the dissociation constants for the parent chemical-HMWM complex, and V_{LI} {L}, the volume of the intracellular space.

1.7.2c Parent chemical in the reservoir in the perfusion system

The rate of change of the parent chemical in the reservoir is simply,

$$RMT_R = Q_L*(ZT_{LS} - ZT_R) \qquad (13)$$

where RMT_R {μmoles/h} is the rate of change of chemical T in the reservoir, Q_L {L/h} is the perfusion flow rate, ZT_{LS} {μM} is the total concentration of T in the outflow from the liver and ZT_R {μM} is the total concentration of T in the reservoir. As discussed in the section on Special Features below, when samples of perfusion medium are collected to determine the concentration of the parent chemical and/or the metabolites, the sample is removed from the reservoir.

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1.7.2d Metabolites

In general, the differential equations describing the behavior of the metabolite in the IPRL system are similar to those for the parent chemical. The differential equations in the reservoir and the liver sinusoidal space are identical. The only difference is in the equation for the metabolite in the intracellular space

$$RMM_{LI} = CM_{LIS}*XM_{LS} - CM_{LSI}*XM_{LI} + RT_{METABOLISM} - RM_{BILE} \qquad (14)$$

where the metabolism term appears with a + sign since the metabolite is being created in the compartment rather than destroyed.

1.7.2e Special note about model code

A multi-component state vector, $DTi(k)$ ($k = 1,2,...,n,n+1$), is used to describe the concentrations of the free and bound species of the parent chemical in the ith compartment. The free concentration is given by the $k = 1$ component, $DTi(1)$, and the remaining n components, $DTi(2)$ to $DTi(n+1)$, describe the concentrations of the n possible ligand complexes. For example, if there is one ligand present in the sinusoidal compartment of the liver that binds the chemical T, the state vector for the sinusoidal compartment has two components:

$$DTLS(1) = XT_{LS} = \text{free parent chemical concentration in the sinusoids}$$
$$\{\mu M\}$$

$$DTLS(2) = YT_{LS} = \text{bound parent chemical concentration in the}$$
$$\text{sinusoids } \{\mu M\}$$

Similar state vectors are used to describe the free and bound concentrations in other compartments - $DTLI(k)$ for the intracellular space of the liver and $DTR(k)$ for the reservoir. Also, similar state vectors are defined for the metabolites, $DMLS(k)$, $DMLI(k)$ and $DMR(k)$. Although the definition of the state variables as vectors appears to introduce unnecessary complexity into the model coding, this approach allows for the introduction of multiple binding ligands without undue additional model coding.

1.8 MODELING MECHANICS AND SPECIAL FEATURES

1.8.1 General modeling mechanics

Model programming was accomplished using ACSL Version 11.4.1 (Advanced Continuous Simulation Language, Mitchel and Gauthier Associates, MA). Executable programs were compiled with the Watcom FORTRAN compiler (v 5.0). All simulations were conducted in the ACSL for Windows environment on a GTSI 486/66 Mhz DX2 desktop computer. Subroutines for solving the nonlinear protein binding equations were programmed in FORTRAN 77.

The general strategy for simulation of the behavior of these simultaneous differential equations is as follows. At the beginning of a time step all free and bound forms of the toxicant are assumed to be in equilibrium. During the time step, the chemical moves across various barriers and flows in and out of the liver compartments under the control of the mass transport differential equations. At the end of the time step, there is a slight displacement from equilibrium due to the change in total chemical concentration in each compartment. The binding equations, using the total mass and binding parameters as inputs, can be solved simultaneously using the DIST11 subroutine to determine the new equilibrium distribution between free and bound species. The new free concentrations then act as the initial conditions for the next time step. The integration algorithm utilized in the implementation of this model is the Gear integration algorithm for stiff ordinary differential equations provided by ACSL.

1.8.2 Special features

The model code includes several features unique to the IPRL system. In most experiments, samples of perfusion medium are collected from the reservoir at specified times during the organ perfusion. Although the total volume of all samples collected is a small fraction of the initial volume of the perfusion medium, corrections must be made for the change in volume and the loss of mass of the parent chemical and metabolites from the system by this process. These corrections are accomplished by creating two s-dimensional vectors, where s is the number of samples collected. The components of the first vector define the times of the samples and the components of the second vector give the volume of the sample collected at the corresponding sample time. The first vector is used to schedule events in the program. When the simulation time equals the sample time, a discrete block of code is called that adjusts the volume of the perfusion medium appropriately and reduced the mass of parent chemical and metabolites by the amount removed. In this manner, corrections for the effects of sampling are made.

A second feature in the IPRL model is a correction for the time delay in the collection of the bile. The transit time for bile to flow from the liver to the collection vial via the biliary cannula is about 9 min. This delay must be incorporated into the model to correct the predicted cumulative mass of parent chemical and metabolites in the bile collected in the interval t_i to t_{i+1} (where t_i and t_{i+1} are successive sampling times). A time delay can be incorporated into the cumulative biliary excretion using a built in function of ACSL. The major impact of the time

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delay is on the first bile fraction collected, i.e., during the first 30 min of exposure to the test chemical. The current version of the IPRL model takes this delay factor into consideration.

2.0 MODEL BEHAVIOR

In order to better appreciate the behavior of the IPRL model, the following specific cases are investigated. Note, in the discussion that follows, the names of state variables and parameters that appear in the model code are used so that the reader will become familiar with the nomenclature and can find the corresponding equations in the program if necessary.

2.1 EFFECT OF VOLUME OF PERFUSION MEDIUM

To investigate the effect of the volume of the perfusion medium (PM) on the kinetics of chemicals in the IPRL, the volume of the PM was varied under the following conditions:

> Membrane transport - simple diffusion, high permeability
> Macromolecular binding - none
> Metabolism - none
> Biliary excretion - none.

This is the simplest case for the IPRL system. As a consequence of the absence of both metabolism and biliary excretion, the system is a closed system with respect to the parent chemical. Furthermore, in the absence of protein binding, metabolism and biliary excretion, and assuming membrane transport is rapid and by simple diffusion, the test chemical will passively distribute between the aqueous phase of the PM and the intracellular water space. The concentration of the parent chemical in the PM at equilibrium will depend on the total water volume (VTOT) of the system. Under the passive conditions assumed for this case,

$$DTR(1)_{t \to \infty} = DTLS(1)_{t \to \infty} = DTLI(1)_{t \to \infty} = \frac{MTDOSE}{VTOT} \tag{15}$$

where $DTR(1)_{t \to \infty}$ {µmoles/L} is the free concentration of the parent chemical in the PM in the reservoir at equilibrium, $DTLS(1)_{t \to \infty}$ {µmoles/L} is the free concentration in the liver sinusoidal space at equilibrium and $DTLI(1)_{t \to \infty}$ {µmoles/L} is the free concentration in the liver intracellular space at equilibrium. Note, in the absence of protein binding, the free concentration is equal to the total concentration in all compartment. For the IPRL system, the total water volume is given by

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$$VTOT = VR + VLS + VLI \tag{16}$$

with VTOT {L} being the total water volume of the IPRL system, VR {L} the volume of the PM in the reservoir and tubing of the system, VLS {L} is the volume of the sinusoidal space in the liver and VLI {L} is the volume of the intracellular water space.

The effect of the volume of PM in the system on the final concentration of the chemical in the PM can be investigated. Assuming a high permeability value (PTLSI*ALSI = PTLIS*ALSI = 10 cm³/sec), so that the chemical in the perfusion medium reaches equilibrium with that in the intracellular water volume rapidly, the kinetic behavior of the system is illustrated in Figure 5A. In Figure 5A, the total concentration (= the free concentration) of the parent chemical T in the PM in the reservoir (ZTR) is plotted for a series of volumes of PM added to the reservoir. Note: For this series of simulations, the dose of the chemical added to the reservoir (MTDOSE) at time t = 0 starts at 10 µmole for the case where VR = 0.200 L and is decreased in proportion to the decrease in the volume of the PM in the reservoir (Table 1). Hence, the initial concentration of the chemical in the PM is constant for all simulations. However, as the volume of the PM added to the reservoir decreases, the total water volume (volume of PM plus water volume of liver) does not decrease in the same proportions. Consequently, the equilibrium concentration of T in the water spaces decreases with decreasing PM volume (Table 1).

The free concentration of T in the hepatic intracellular water space (DTLI(1)) is illustrated in Figure 5B. Because the permeability of the hepatic sinusoidal membranes is high, the intracellular concentration rapidly attains equilibrium with the concentration in the PM.

The average concentration of the chemical in the liver, AVGTL {µmoles/Kg}, is given in Figure 5C. The average liver concentration is less than the intracellular concentration due to the fact that the chemical is assumed to distribute only into the water phase of the tissue, and not to distribute into either the lipid compartment of the liver or the solid phase (connective tissue). Thus, when computing the tissue average concentration, the weight of these compartments has a noticeable influence since they contribute to the denominator but not the numerator. Under these conditions (closed system - rapid equilibration), the average liver concentration at any time can be predicted from the concentration in the PM:

$$AVGTL = \frac{MTL}{WL} = \frac{DTLS(1)* VLS + DTLI(1)* VLI}{WL} \tag{17}$$

23

A
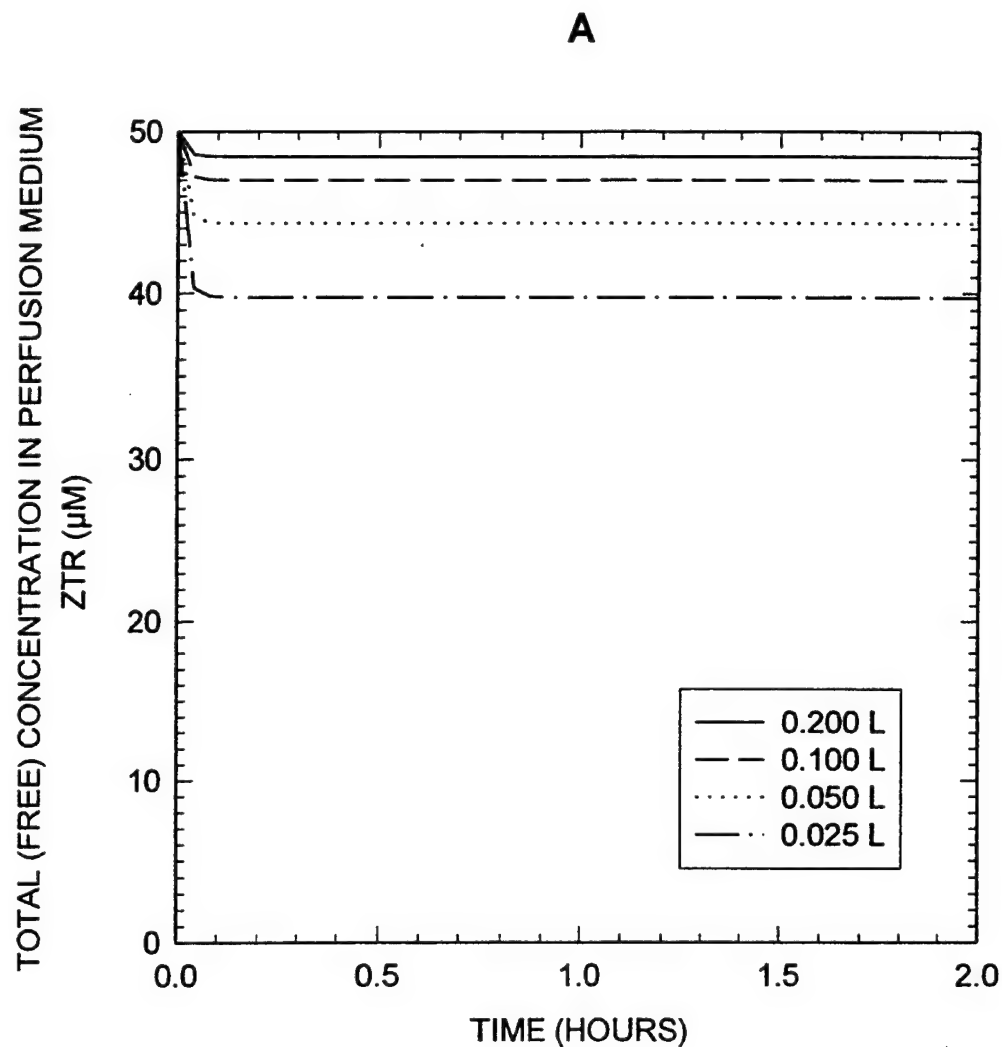
Figure 5: Effect of Volume of Perfusion Medium on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) total (free) concentration of the parent chemical in the intracellular water space (DTLI(1)), (C) average concentration of parent chemical in the liver (AVGTL). VR varies from 0.200 to 0.025 L.

24

B

C

Figure legend:
- —— 0.200 L
- — — 0.100 L
- ·········· 0.050 L
- —·—· 0.025 L

Y-axis: AVERAGE LIVER CONCENTRATION AVGTL (µMOLES/KG)

X-axis: TIME (HOURS)
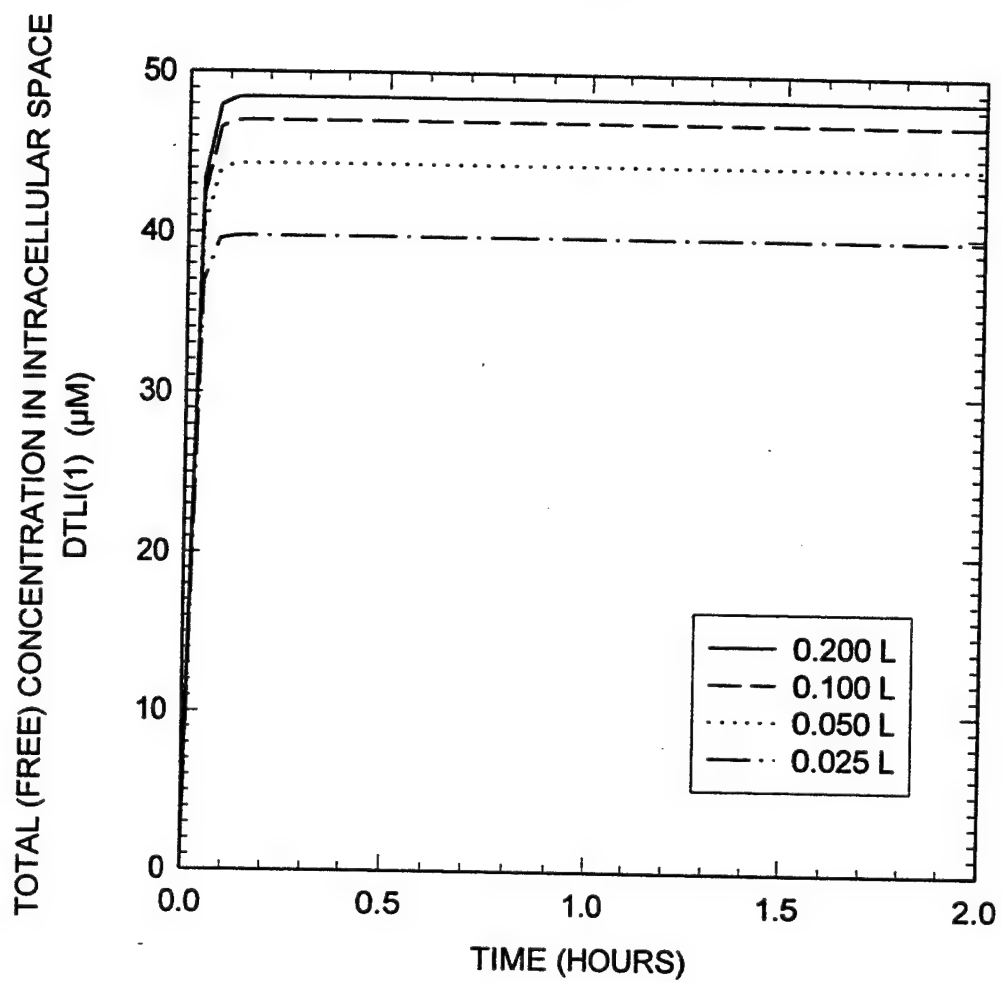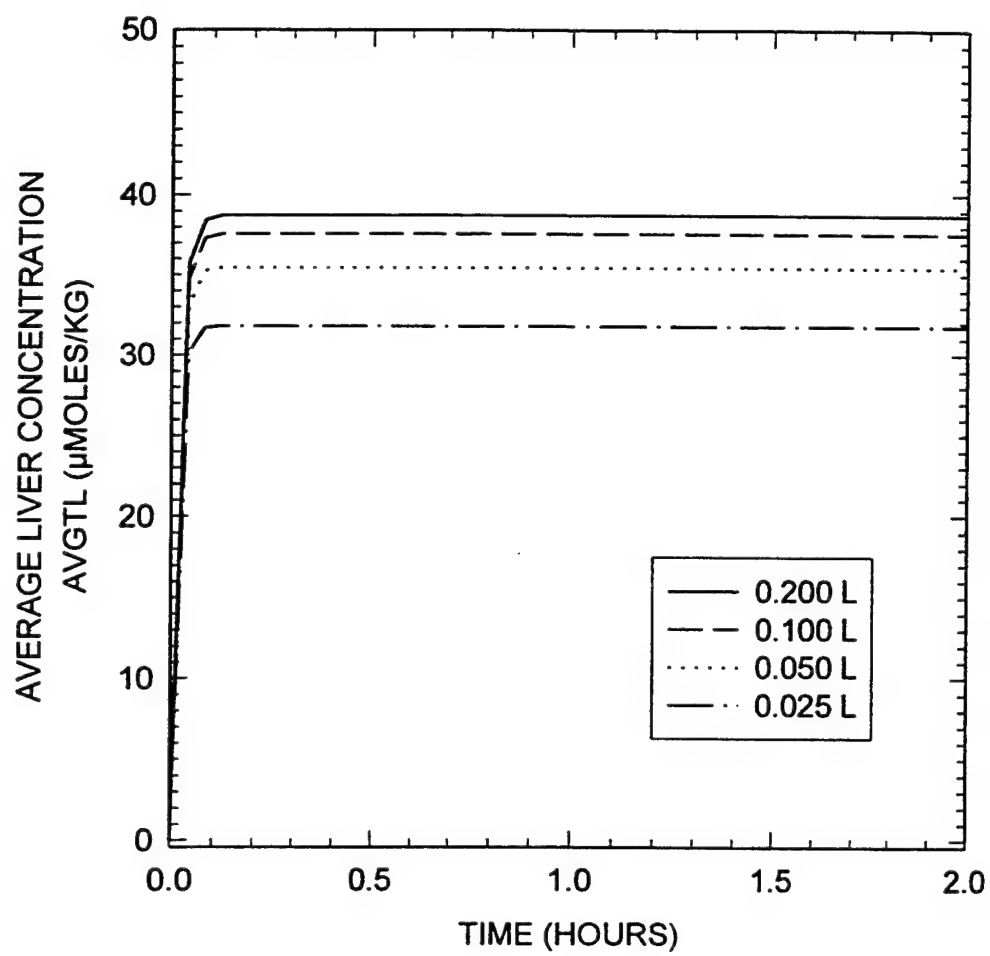
Volume of PM VR (L)	Dose of Test Chemical MTDOSE (µmoles)	Total Water Volume of System* VTOT (L)	Free Concentration of T in PM	
			DTR(1)$_{t=0}$ (µM)	DTR(1)$_{t\to\infty}$ (µM)
0.200	10.0	0.2064	50.0	48.4
0.100	5.0	0.1064	50.0	47.0
0.050	2.5	0.0564	50.0	44.3
0.025	1.25.	0.0314	50.0	39.8

* Assuming a liver weight of 0.008 Kg and corresponding volumes of VLS = 0.0018 L and VLI = 0.0046 L.

Table 1: Effect of volume of PM added to the reservoir on the equilibrium free concentration of the test chemical in the PM (DTR(1)$_{t\to\infty}$). The dose is scaled in proportion to the decrease in the volume of PM to assure that the initial concentration is the same in all cases.

where MTL {µmoles} is the total chemical in the liver and WL is the weight of the liver. At equilibrium, Equation (15) can be used to reduces this to

$$AVGTL_{t\to\infty} = DTR(1)_{t\to\infty} * \left(\frac{VLS + VLI}{WL} \right) \tag{18}$$

Thus, the ratio of the average liver concentration to the concentration in the PM at equilibrium, i.e., the "partition coefficient" is

$$\frac{AVGTL_{t\to\infty}}{DTR(1)_{t\to\infty}} = \frac{VLS + VLI}{WL} = 0.78 \tag{19}$$

Any significant deviation from this ratio would indicate that the system is not behaving in a passive manner and that other factors are contributing to the distribution of T between the PM and the tissue. This last statement assumes that the system has attained equilibrium, which can be ascertained from the kinetics of T in the PM.

Note that the system is linear under the assumptions invoked for this case. Therefore, for any given volume of PM added to the system, the state variables describing the behavior of the chemical in the system scale in proportion to the

27

dose. If the dose is doubled, the concentration in the PM will double, the intracellular concentration will double and the average concentration in the liver will double. The proportional scaling of the response in relation to the dose is a characteristic of a linear system. This behavior can be used to test the linear condition.

2.2 EFFECT OF MEMBRANE TRANSPORT ON KINETICS

2.2.1 Simple Diffusion

To investigate the effects of diffusional permeability on the kinetics of a chemical in the IPRL system, the permeability*area product was varied under the following conditions:

Membrane transport - simple diffusion, diffusional permeability variable
Protein binding - none
Metabolism - none
Biliary excretion - none

In the case discussed above (Section 2.2), the effect of changing the volume of the perfusion medium on the equilibrium concentration of T in the PM was investigated. In the simulation runs, the permeability of the hepatic sinusoidal membrane was set to a high value in order to reach equilibrium rapidly between the PM and the intracellular water space. The rate of the distribution process, i.e., the time it takes to reach equilibrium, will be controlled by the permeability of the hepatic sinusoidal membrane. In principle, the permeability of this membrane can vary over a large range. The effects of permeability on the kinetic behavior of T in the PM is illustrated in Figure 6A. In this case, the volume of PM is 0.200 L and the liver weight is 0.008 Kg. A dose of 10 μmoles of chemical is added and the permeability*area product is allowed to vary from 0 to 10 cm³/sec.

If the permeability of the hepatic sinusoidal membrane is zero, i.e., the chemical cannot pass across the membrane and enter the hepatocytes, the concentration of T in the PM is constant after the transient mixing period (the time required for the chemical to pass through the perfusion system and flush out the sinusoidal spaces of the liver and the tubing in the system, ~ 30 sec). The concentration attained is simply the dose (MTDOSE {μmoles}) divided by the water volume of the system, excluding the hepatic intracellular water volume (VR + VLS, {L}), or

28

A
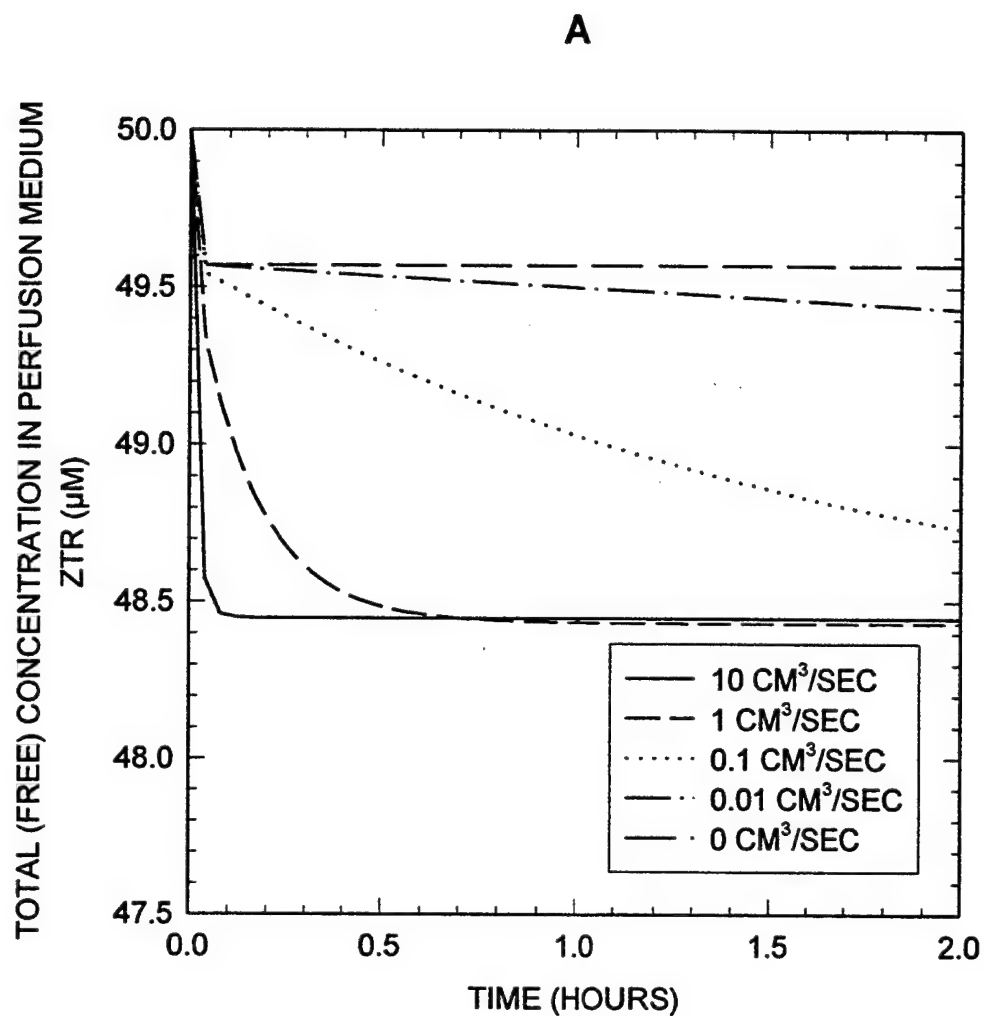
Figure 6: Effect of Diffusional Permeability on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) total (free) concentration of the parent chemical in the intracellular water space (DTLI(!)), (C) average concentration of parent chemical in the liver (AVGTL). ALSI*PTLIS = ALSI*PTLSI varies from 0 to 10.0 cm^3/sec.

29

B

C

31
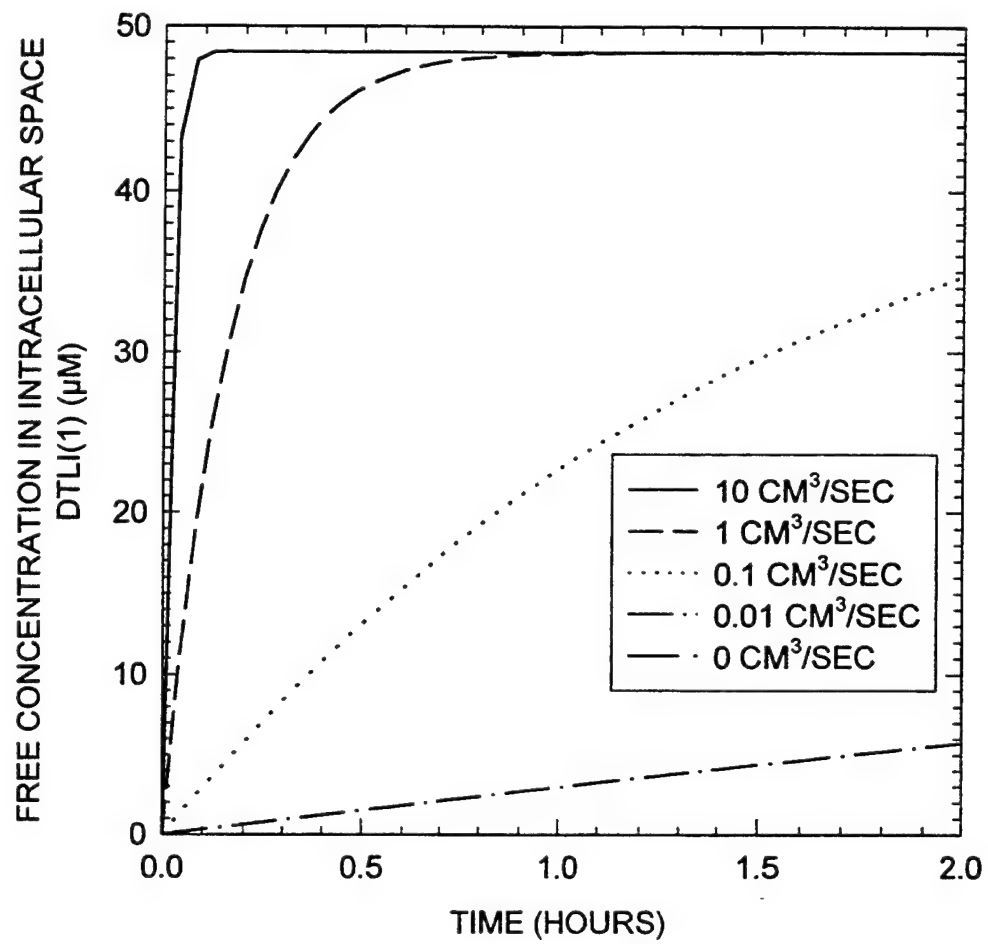
$$DTR(1)_{t \to \infty} = \frac{MTDOSE}{VR + VLS} \tag{20}$$

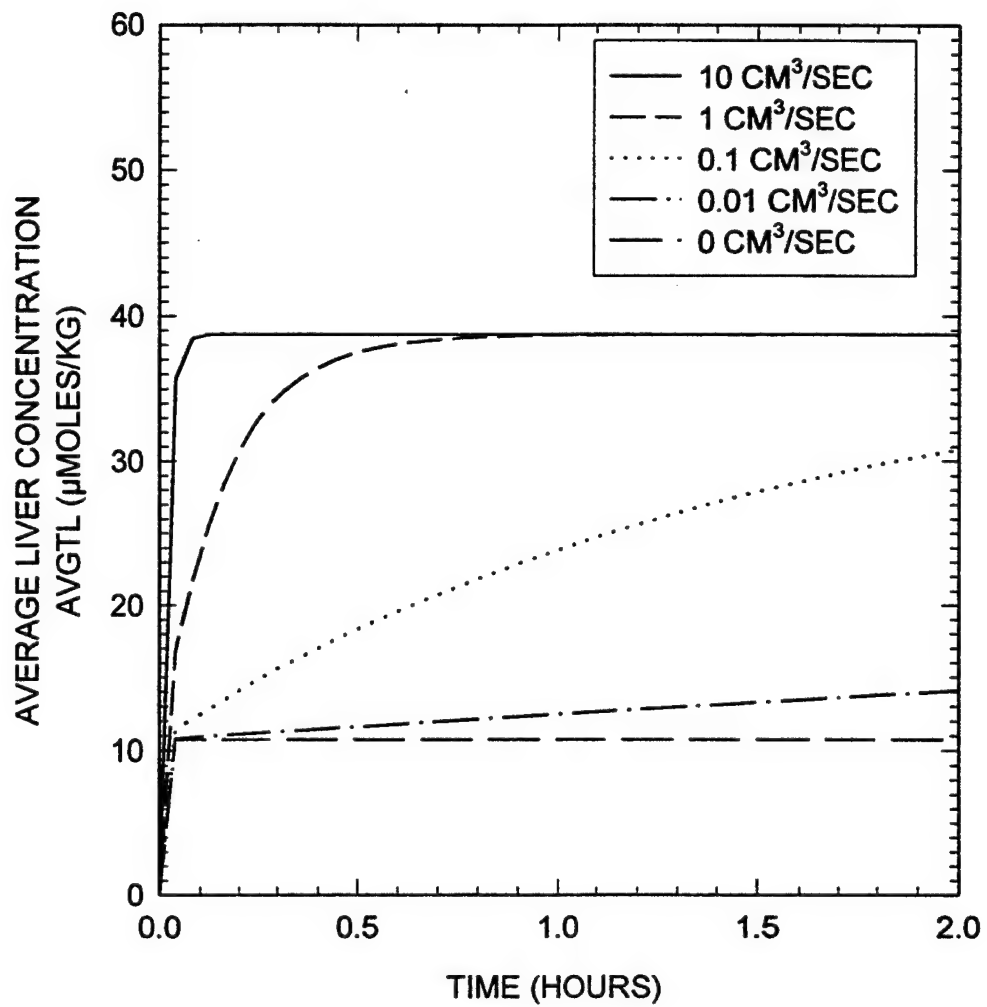The duration of the initial mixing process will depend on the perfusion rate, QL = 2.4 L/h, and the volume of the tubing in the perfusion apparatus. In Figure 6A, the initial mixing accounts for the rapid drop in concentration in the PM from 50.0 to 49.6 µM during the first 2 minutes.

If the permeability of the S-I interface is greater than zero, the test chemical can penetrate into the intracellular water space and the test chemical distributes into the volume of distribution, i.e., the total water volume of the system (VTOT). The rate at which equilibrium is attained between the PM and the intracellular water volume increases with increasing permeability, i.e., the time to equilibrium decreases. This is seen in Figure 6A. At low permeability, PA = 1.0 x 10^{-2} cm³/sec, the system does not reach equilibrium during the two hour duration of the standard perfusion experiment. Whereas, at high permeability (PA = 10 cm³/sec), equilibrium is attained within minutes. In all cases, except PA = 0, the final concentration, given by Equation (15), is the same, however, it is attained faster as the permeability increases.

The intracellular free concentration, DTLI(1), is illustrated in Figure 6B. When the permeability of the sinusoidal plasma membrane to the test chemical is zero, the concentration of T in the intracellular water space is also zero because no test chemical can penetrate into this space. As the permeability increases, the test chemical has access to the intracellular water space and the intracellular concentration increases to the equilibrium level given by Equation (15). As described above, the rate at which equilibrium is attained is related to the permeability.

The average concentration of T in the liver is illustrated in Figure 6C. If the permeability is zero, the intracellular concentration of T is zero as a consequence of the fact that the chemical cannot enter the cell. However, the liver average concentration is not zero due to the presence of chemical in the sinusoidal space. In this case, the average concentration of T in the liver is equal to the amount of chemical in the PM retained in the liver sinusoids divided by the liver weight

$$AVGTL = \frac{DTLS(1)*VLS}{WL} \tag{21}$$

which is only about 1/5th the concentration in the PM (VLS/WL ≈ 0.22), as indicated in Figure 6C. If the permeability is non-zero so that the test chemical can penetrate into the intracellular space, then at equilibrium AVGTL will be given by Equation (18). **Note:** if the sinusoidal space in the liver is flushed out with chemical

free buffer at the end of the experiment, the calculation of AVGTL must be corrected for the chemical removed.

This system is also a linear closed system. Thus, the response of system variables, such as concentrations, will scale in proportion to the dose.

### 2.2.2 Mediated Transport (Facilitated Diffusion / Active Transport)

Mediated transport involves molecular carriers that provide a pathway for transport of lipid insoluble chemicals across cellular membranes. The major characteristic of mediated transport is the fact that there is a maximum rate for transport, and when the transport system is saturated, the rate of transport becomes zero order, i.e., independent of concentration. In the IPRL model, mediated transport can function in both directions at the S-I interface. If the process is symmetrical, i.e., transport parameters are identical for transport in either direction, then the model simulates facilitated diffusion. If the transport process is asymmetrical, then the model simulates active transport.

To investigate the behavior of mediated transport processes, the follow conditions were simulated:

Membrane transport - facilitated diffusion, vary maximum rate
Protein binding - none
Metabolism - none
Biliary excretion - none

Figure 7A illustrates the concentration of the parent chemical in the perfusion medium as the maximum rate for facilitated diffusion (ALSI*TTTLSI = ALSI*TTTLIS) was varied from 0 - 1000 nmoles/sec. The dose used for this simulation was 10 µmoles and the the concentration for half-maximum transport rate (KTTLSI = KTTLIS), was 10 µM. As the maximum rate increases, the system reaches steady state more rapidly. The final steady state is attained when the free concentration of the chemical in the intracellular space is equal to the free sinusoidal concentration. Note the final steady state concentration is the same as that observed when the transport mechanism was simple diffusion (see above). This is due to the symmetical, passive nature of facilitated transport. This mechanism is important for water soluble, lipid insoluble chemicals that would not ordinarily penetrate into cells in the absence of a mediated transport process (e.g., sugars, amino acids).

The facilitated diffusion process is not obviously different from simple diffusion unless the effect of saturation is explored. To do this, it is necessary to investigate a range of doses that results in chemical concentrations that overlap
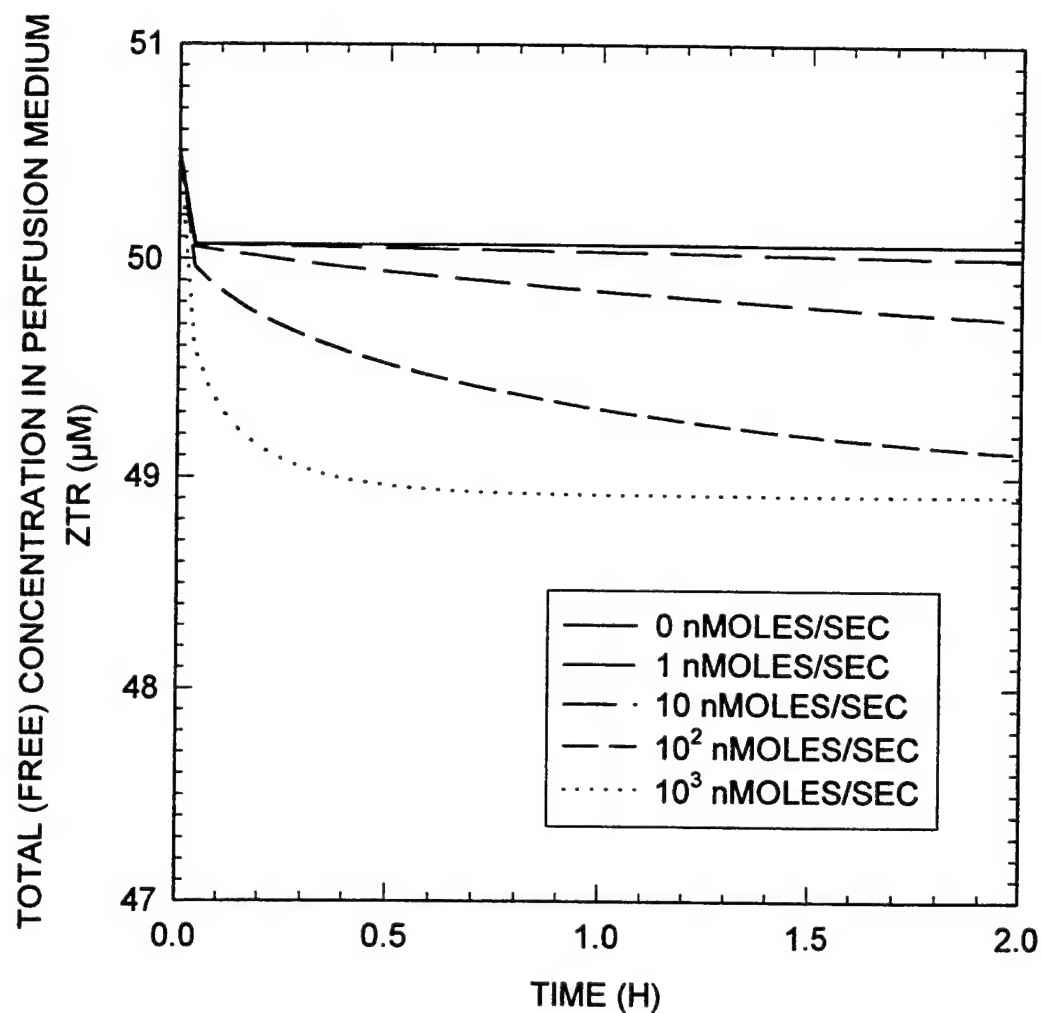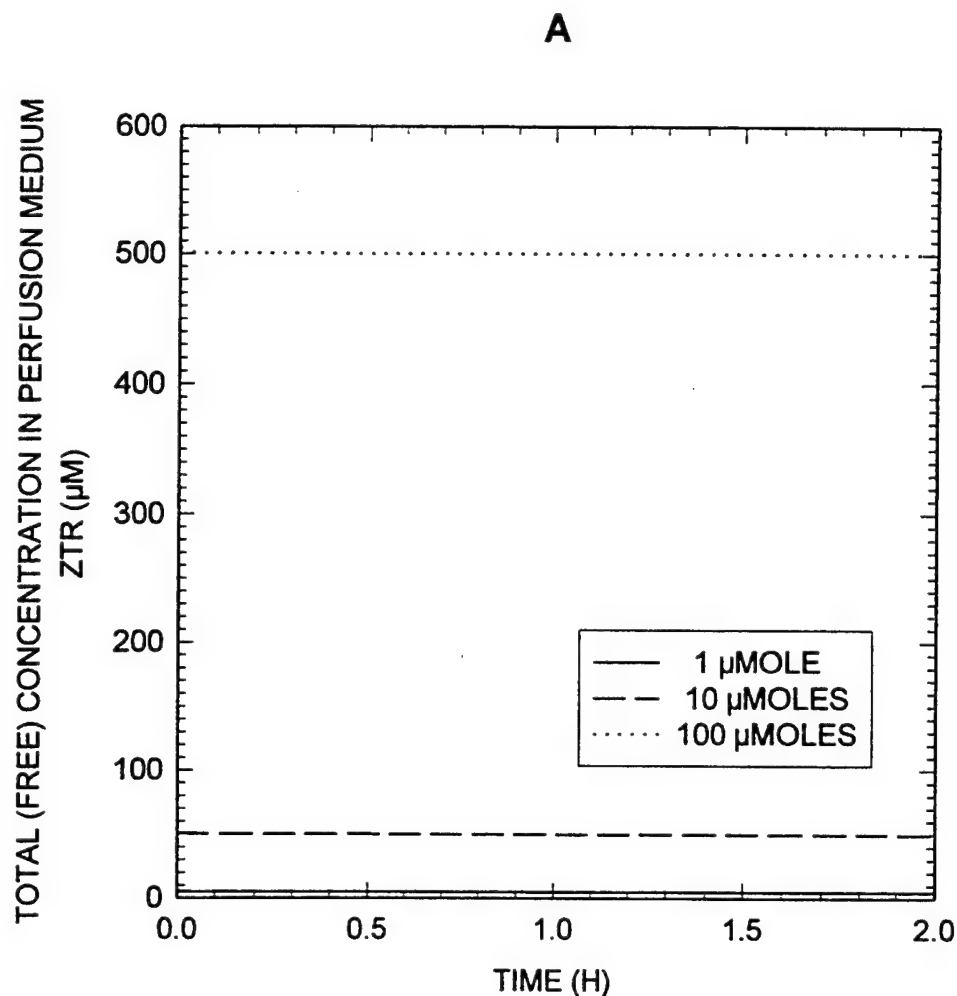
Figure 7: Effect of Facilitated Diffusion on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

Total (free) concentration of the parent chemical in the PM (ZTR). ALSI*TTTLIS = ALSI*TTTLSI varies from 0 to 1000 nmoles/sec.

the KTTLIS (= KTTLSI). Using doses ranging from 1 - 100 µmoles, the effects of saturation on the chemical kinetics can be observed. Figure 8A illustrates the concentration of the chemical in the perfusion medium over this range of doses. These data are unremarkable and would not allow one to discriminate between simple and facilitated diffusion. Similarly, the data for the final liver concentration, AVGTL (Figure 8B), does not show an obvious effect. This apparent lack of effect is due to the fact that the major influence of saturation of the transport process shows up in the rate at which the intracellular compartment reaches steady state while the total liver concentration is influenced mainly by the chemical present in the perfusion medium trapped in the sinusoidal space. The only experimental way to see the saturation effect clearly is to plot the final liver concentration versus dose (Figure 8C). Now the effect of saturation of the transport process is revealed.

It is also interesting to evaluate the effect of changing the apparent affinity of the carrier, KTTLSI = KTTLIS, for a given non-rate limiting maximum transport rate. Setting ALSI∗TTTLSI = ALSI∗TTTLIS = 10^3 nmoles/sec, and letting KTTLSI = KTTLIS vary from 1000 - 10 µM for a fixed dose (MTDOSE = 10 µmoles), it is observed that when the transporter "affinity" is low (KTTLij = 1000 µM), the transport system is functioning suboptimally (Figure 9A - parent chemical concentration in PM and Figure 9B - average liver concentration). As the affinity increases, the transport system operates more efficiently and the maximum transport rate becomes the limiting factor.

Finally, as an example of the possible description of active transport, we can make the mediated transport process asymmetrical. Setting the affinity equal for transport in either direction, KTTLSI = KTTLIS = 10 µM, and the maximum transport rate from the intracellular to the sinusoidal space at a high non-rate-limiting value, ALSI∗TTTLSI = 10^3 nmoles/sec, the maximum inward transport rate, ALSI∗TTTLIS, can be varied from 10^3 - 10^4 nmoles/sec. In Figure 10A the effects of asymmetrical mediated transport on the concentration of the parent chemical in the PM is illustrated. For highly asymmetical transport parameters, that favor chemical transport into the liver intracellular space, it is seen that the chemical is rapidly depleted in the PM. Figure 10B shows the effect on the average liver concentration. The limitation on the total liver concentration occurs when almost all of the parent chemical is transported from the perfusion medium into the liver. If the available chemical was not depleted in the perfusion medium, the total liver concentration would continue to increase with increasing asymmetry. Note: In the case studied, the ratio of the intracellular free concentration to sinusoidal free concentration dose increase even at high asymmetry when the total liver concentration does not appear to increase. Figure 10C shows the intracellular concentration of the parent chemical. At steady state, the ratio of chemical in the intracellular space to the perfusion medium reflects the ratio of the forward to reverse maximum transport rates constants (ALSI∗TTTLIS:ALSI∗TTTLSI). This

35

A

Figure 8: Interactive Effect of Dose and Mediated Transport on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) average concentration of parent chemical in the liver (AVGTL), and (C) dose dependency of the average liver concentration at the end of the experiment (AVGTL(t=120 min). MTDOSE varies from 1 to 100 μmoles.

B

C

Y-axis: AVERAGE LIVER CONCENTRATION AVGTL (µMOLES/KG)

X-axis: MTDOSE (µMOLES)

A
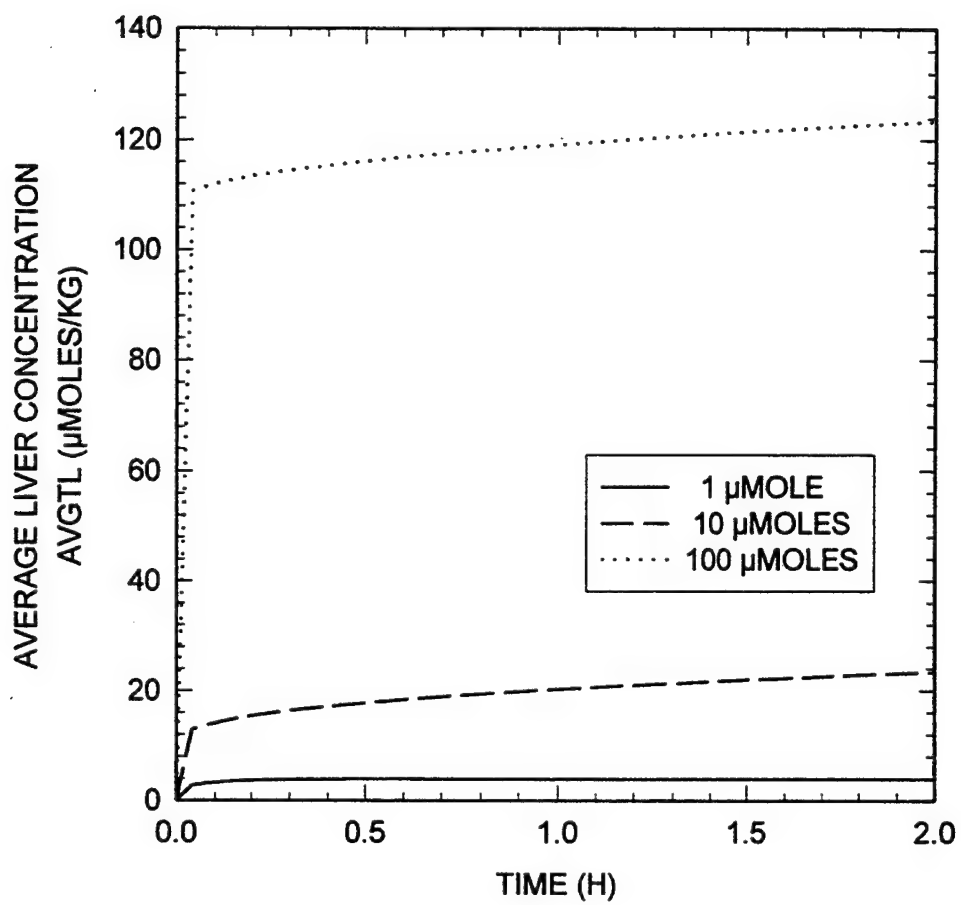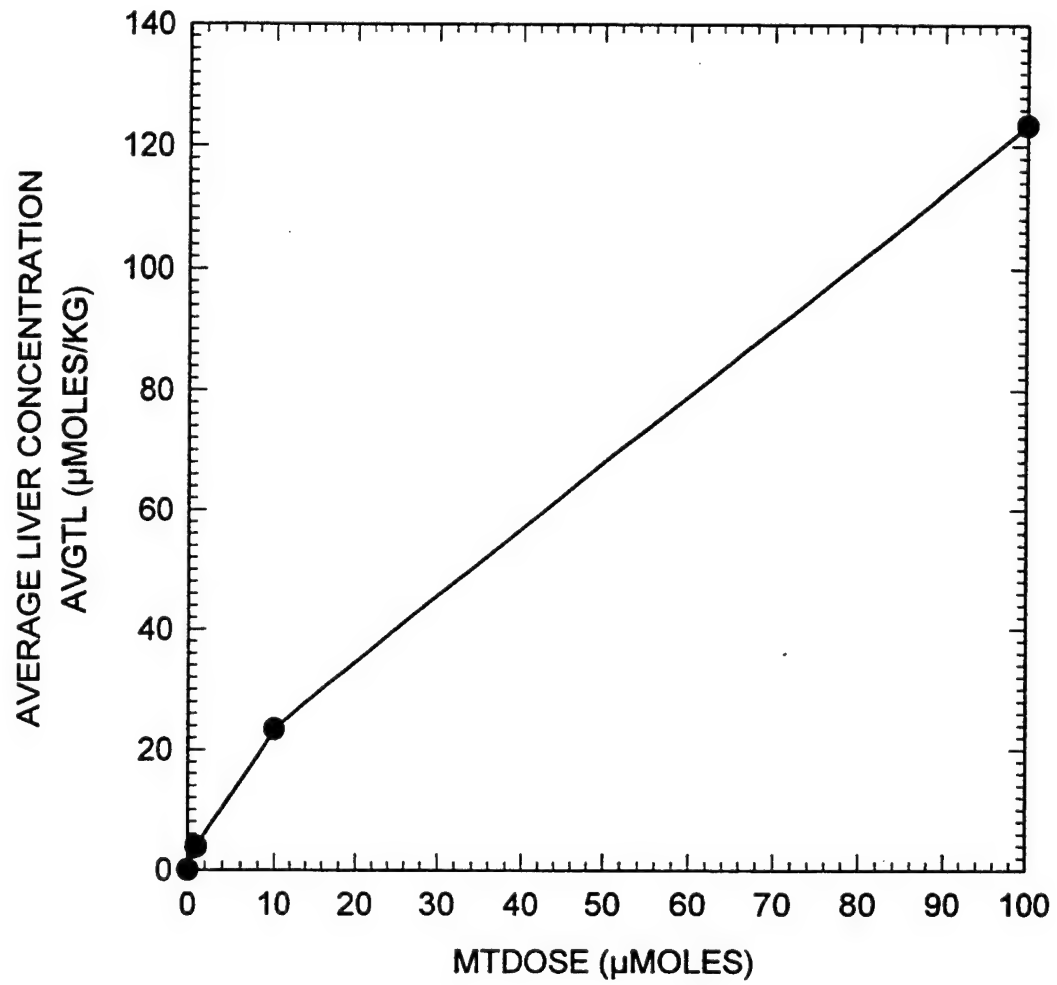
**Figure 9: Effect of KTTLSI (= KTTLIS) on the Kinetics of the Parent Chemical
T in the Isolated Perfused Rat Liver System.**

(A) Total (free) concentration of the parent chemical in the PM (ZTR) and, (B)
average liver concentration of parent chemical (AVGTL). KTTLSI = KTTLIS varies
from 1000 to 10 µM.

B

A

Figure 10: Effect of Assymetrical Mediated Transport on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) average concentration of parent chemical in the liver (AVGTL), and (C) total (free) concentration of the parent chemical in the intracellular water space (DTLI(1)). ALSI*TTTLSI = 10^3 nmoles/sec, ALSI*TTTLIS varies from 10^3 to 10^4 nmoles/sec.

41

B

C

FREE CONCENTRATION IN INTRACELLULAR SPACE DTLI(1) (µM)

TIME (H)
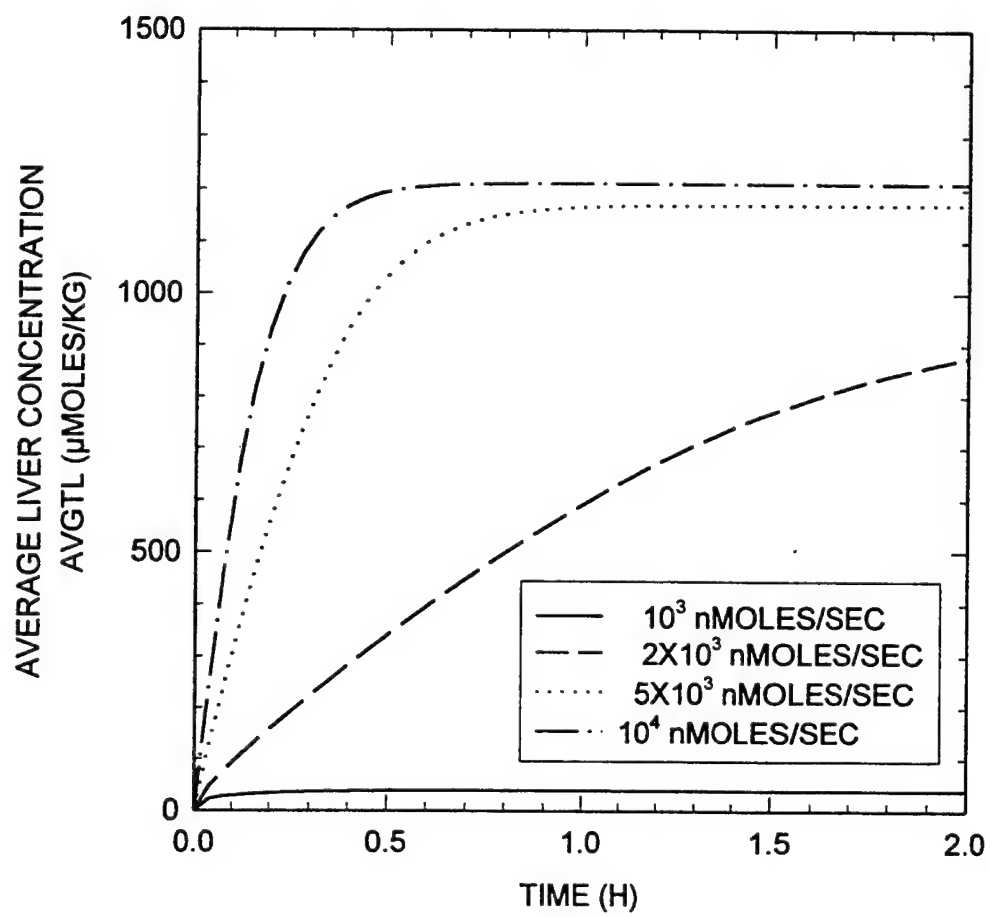
Legend:
- 10^3 nMOLES/SEC
- 2×10^3 nMOLES/SEC
- 5×10^3 nMOLES/SEC
- 10^4 nMOLES/SEC

exercise illustrates how active transport can significantly influence the distribution of a chemical within the IPRL system.

2.3 EFFECT OF INTRACELLULAR MACROMOLECULAR BINDING

To investigate the influence of reversible protein binding on chemical kinetics, the effect of intracellular binding will be explored first. Both the capacity and the affinity of the intracellular ligand will be evaluated.

2.3.1 Binding Capacity

The effect of varying the capacity of the intracellular ligand was evaluated under the following conditions:

Membrane transport - simple diffusion, high permeability
Macromolecular binding - intracellular binding, vary UBTLI
Metabolism - none
Biliary excretion - none

Note: A special case exists if the permeability of the sinusoidal plasma membrane is zero, PA = 0 cm³/sec. In this situation, intracellular macromolecular binding has no effect since the chemical does not penetrate into the intracellular space. However, if the permeability of the membrane is non-zero, then intracellular macromolecular binding can have a profound effect on the ultimate equilibrium distribution of the test chemical.

To illustrate the effects of intracellular macromolecular binding, the condition with MTDOSE = 10 µmoles and ALSI∗PTLSI = ALSI∗PTLIS = 10 cm³/sec (high permeability) has been simulated. The concentration of the free chemical in the PM is illustrated in Figure 11A for various values of the total binding capacity in the intracellular space (ranging from UBTLI = 0 to 10,000 µM). It is assumed that the binding affinity of the intracellular ligand is moderate (KBTLI = 10 µM). As intracellular binding increases, the equilibrium concentration in the PM decreases as more of the chemical is sequestered as the bound complex in the intracellular space. At very high intracellular binding capacities, equilibrium is not attained during the course of the standard 2 hour experiment. Even though the permeability of the sinusoidal plasma membrane is relatively high, the accumulation of the chemical in the intracellular water space, as the sequestered bound species, is limited by the transport of the chemical. In this particular case, if the experiment was extended to allow equilibrium to be established, then the free concentration in the PM would be quite low and the majority of the chemical would be found bound in the liver.
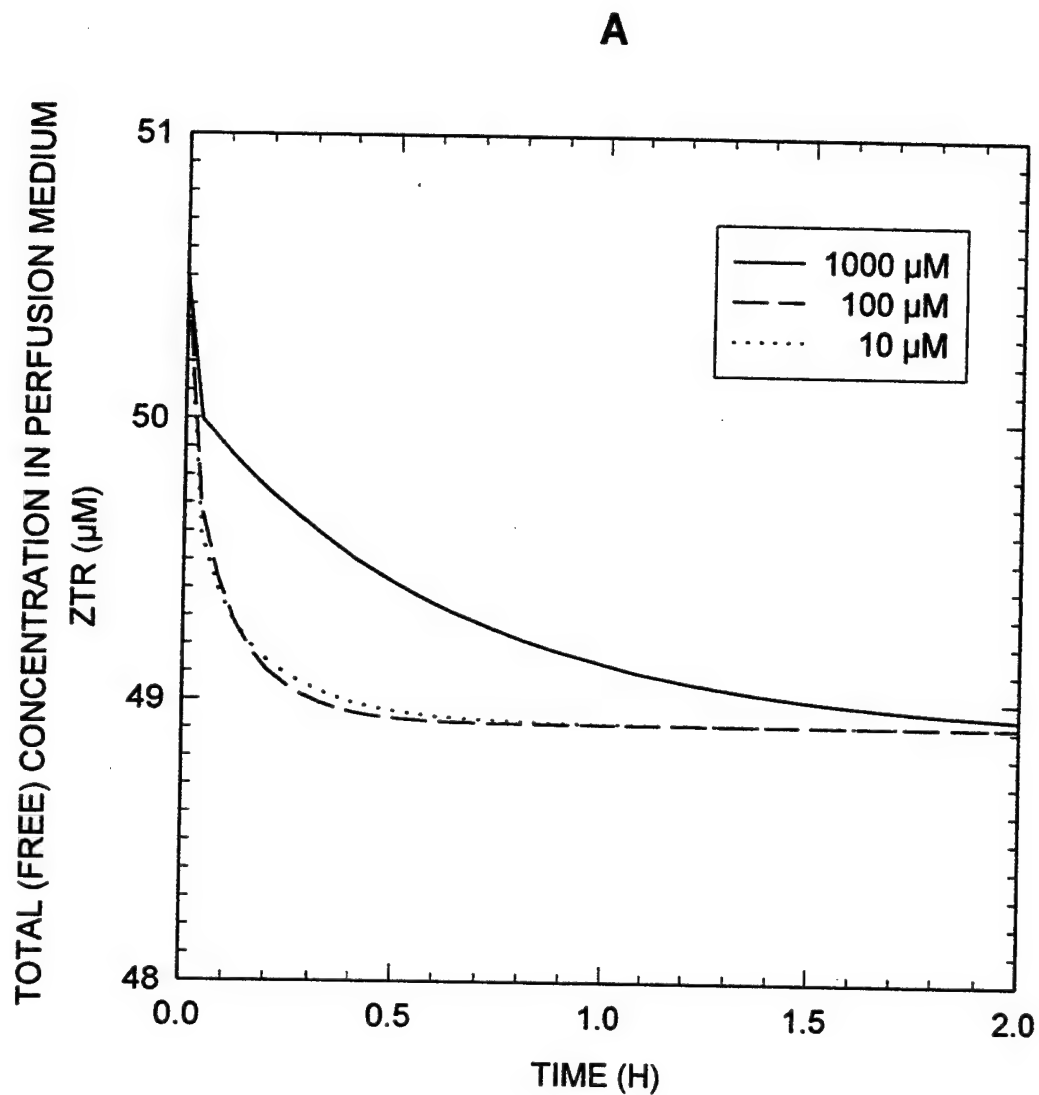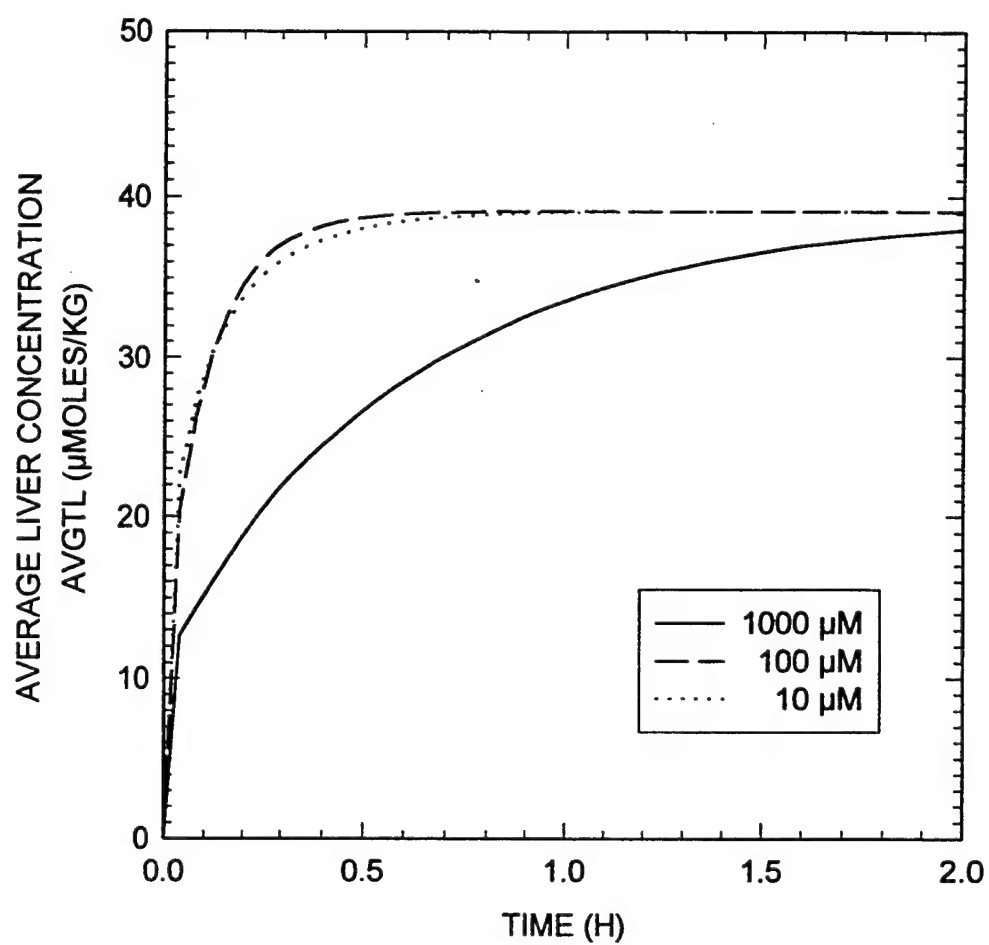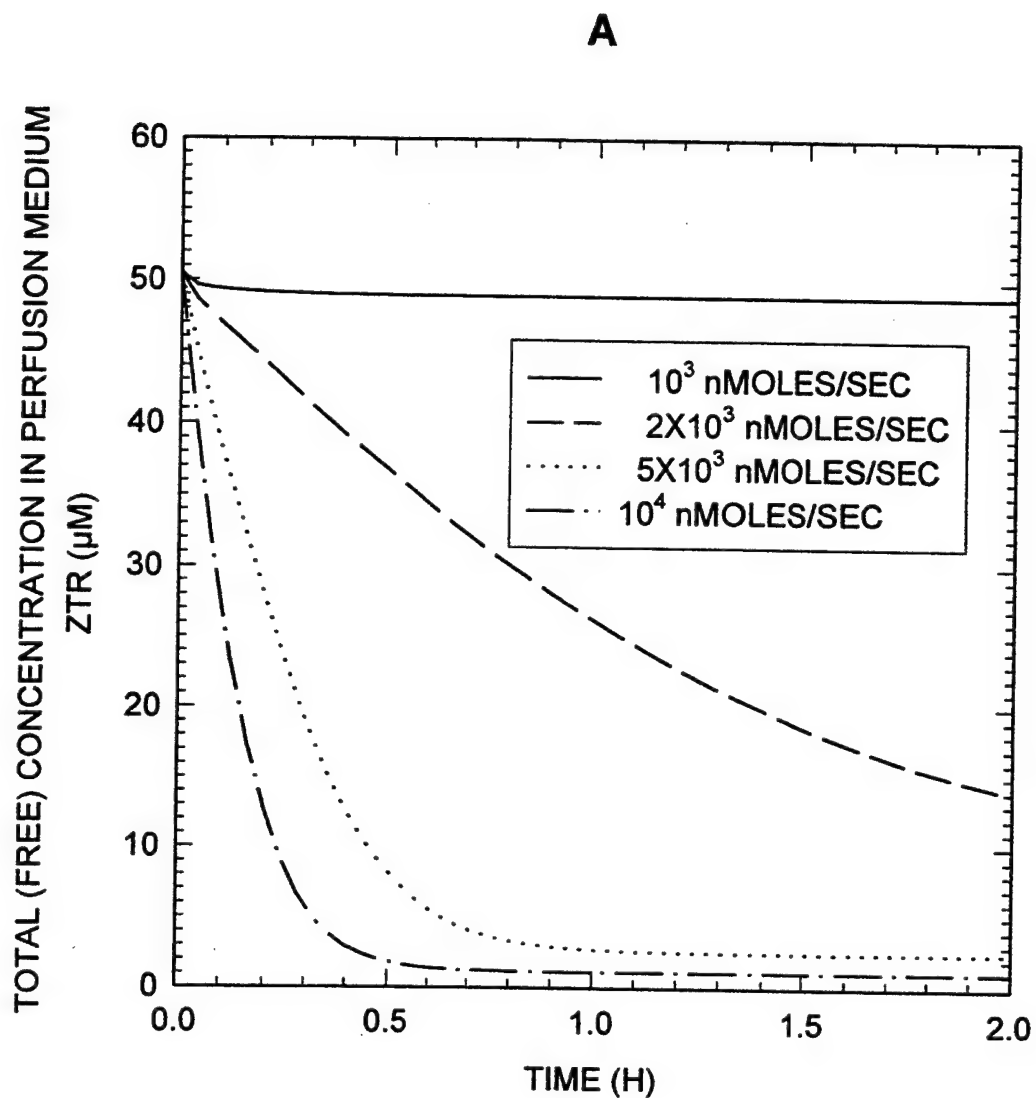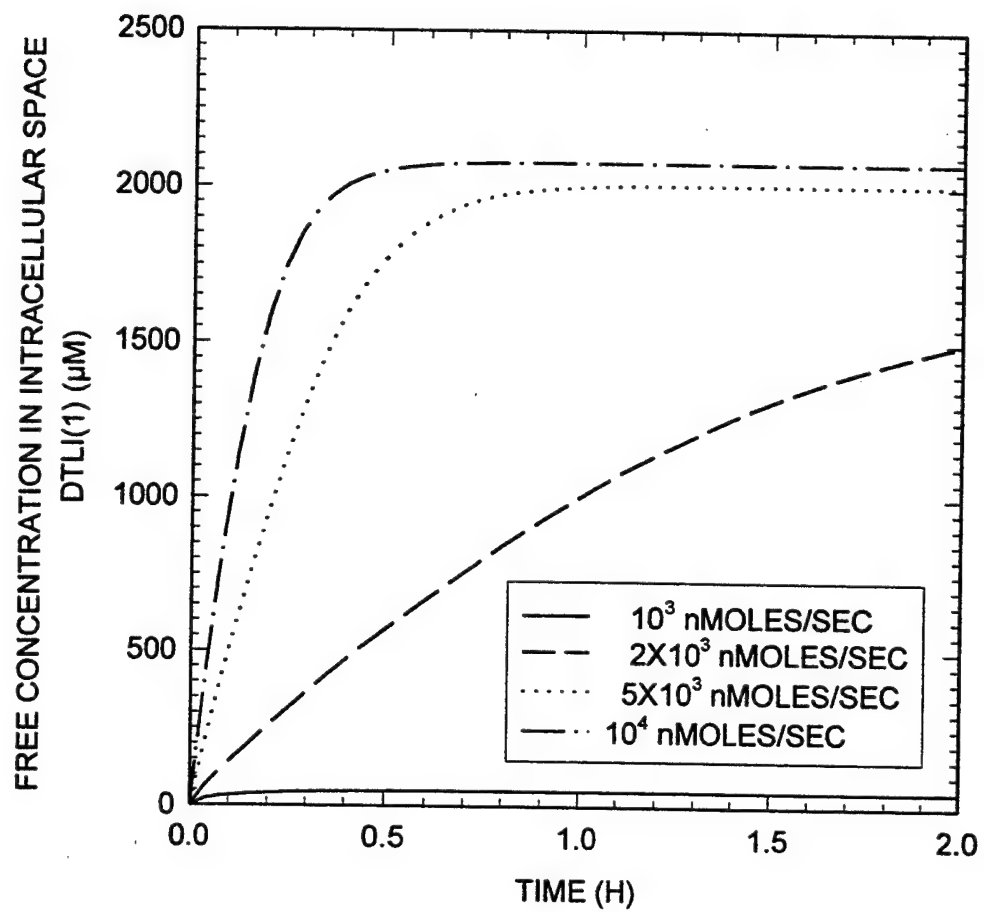
44

A
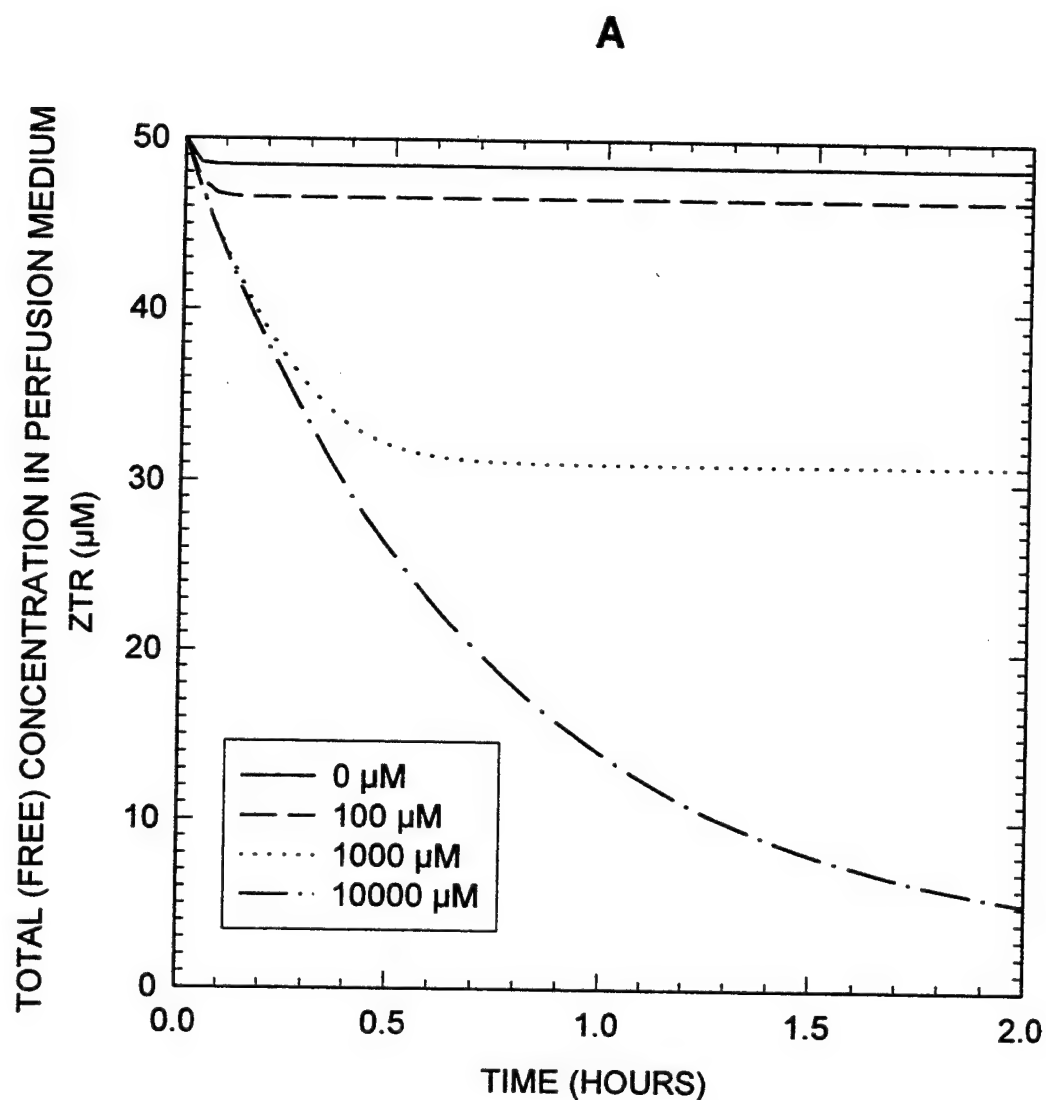
Figure 11: Effect of Intracellular Binding on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) total (free) concentration of the parent chemical in the intracellular water space (DTLI(1)), (C) bound concentration of parent chemical in intracellular space (DTLI(2)), and (D) average concentration of parent chemical in the liver (AVGTL). UBTLI varied from 0 to 10^4 µM

B

C

Figure with y-axis labeled "BOUND CONCENTRATION IN INTRACELLULAR SPACE DTLI(2) (µM)" ranging from 0 to 2000, and x-axis labeled "TIME (HOURS)" ranging from 0.0 to 2.0. Legend shows: 0 µM, 100 µM, 1000 µM, 10000 µM.

47

D
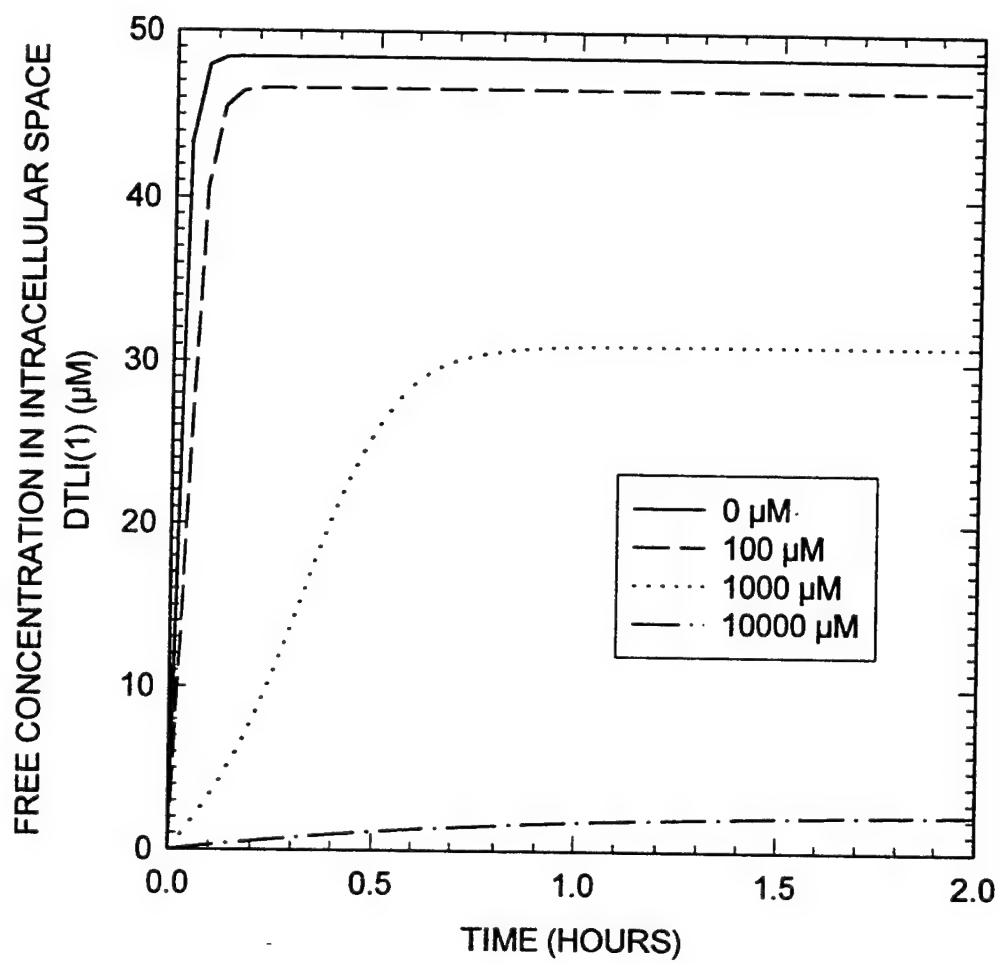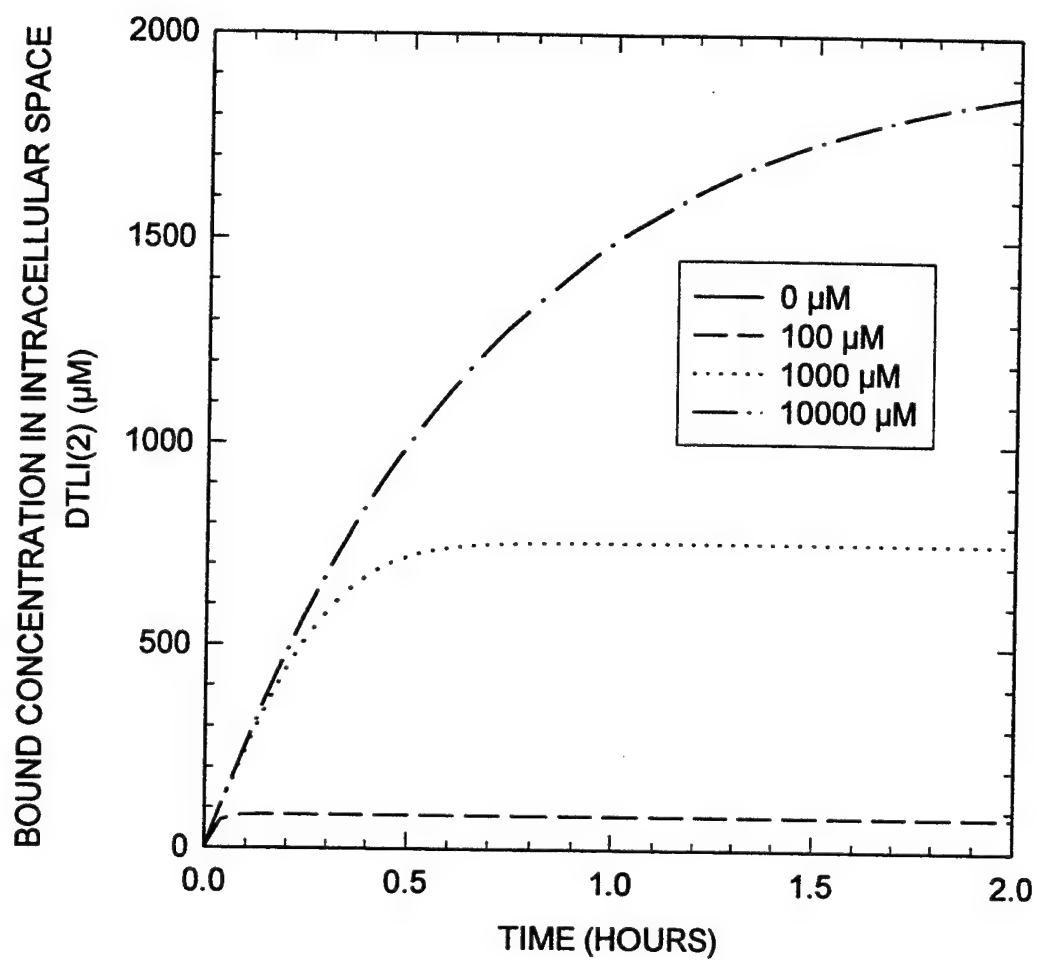
The free and bound concentrations of T in the intracellular water space is illustrated in Figures 11B and C, respectively. As the binding capacity increases, the free concentration decreases since a larger portion of the chemical is sequestered as the bound species. The importance of intracellular binding is two fold. First, more of the chemical is retained in the tissue and this may be of concern from a toxicological point of view. If the binding is to a molecular target that causes toxicity, this effect will have a negative impact on cellular survival. On the other hand, if the binding is to a non-functional site in the tissue, then the effect will be beneficial and result in detoxication. Thus, it is possible to have a higher concentration in the tissue but less toxicity if the macromolecular binding is a detoxication mechanism. Second, the chemical bound to intracellular macromolecules is not directly available for other kinetic processes. Thus, macromolecular binding can influence the rate of metabolism of the parent chemical and biliary excretion (see below). In Figure 11C, it is observed that as the intracellular binding capacity increases the concentration of the bound species in the intracellular water space increases.

The average tissue concentration, AVGTL, increases with increasing binding capacity in the intracellular water space and is illustrated in Figure 11D. The absolute upper limit to the tissue concentration would occur when all the test chemical was sequestered as the bound species in the intracellular water space, i.e., if the intracellular ligand had a very high affinity and capacity. In the example here, the limiting concentration would be

$$AVGTL_{MAX} = \frac{MTDOSE}{WL} = 1250 \ \mu MOLES \ / \ KG \tag{22}$$

Macromolecular binding is a non-linear process. Therefore, in the presence of macromolecular binding the system becomes non-linear. This implies that as the dose increases, the response of state variables will not change in the same proportions. The main effect will be clearly seen in the 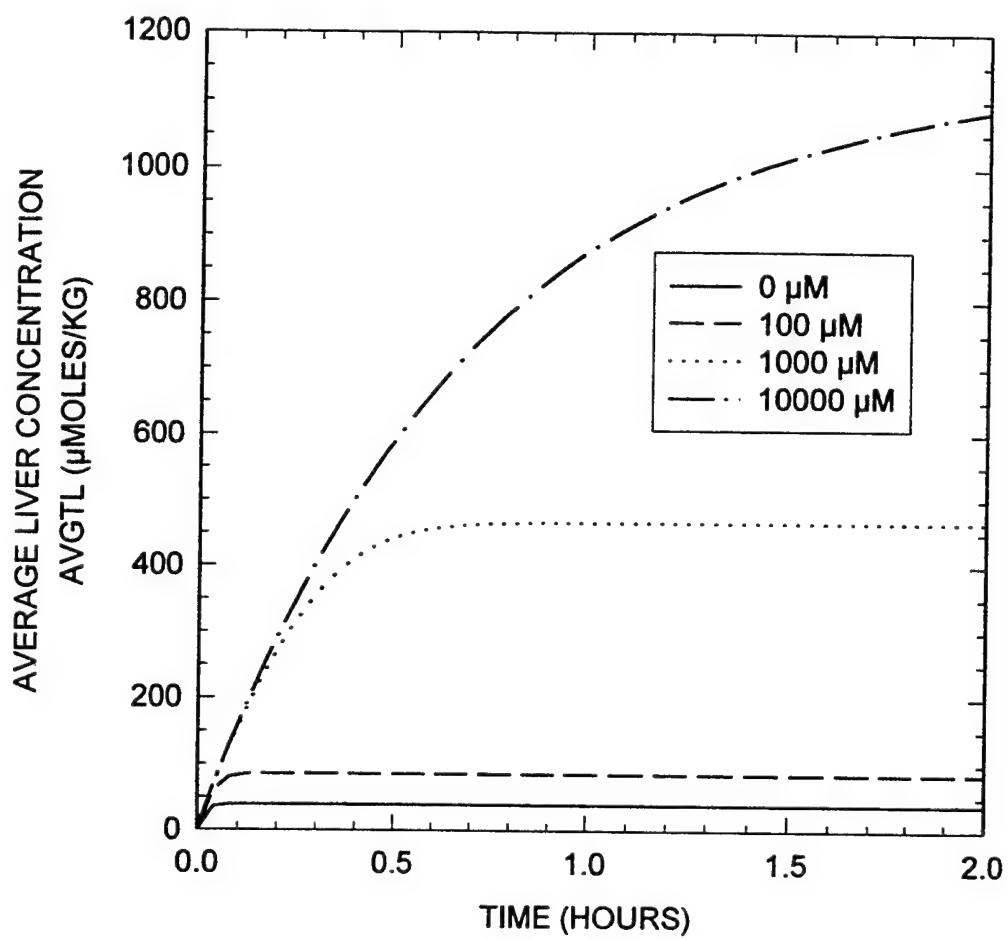average liver concentration as a consequence of the fact that at a sufficiently high dose, the intracellular binding capacity will be saturated. At even higher doses, additional chemical, above and beyond that which is required to saturate the ligand, will distribute within the system as free chemical. Thus, the proportions of test chemical in the tissue and the PM will shift to more in the PM at higher doses. This effect can be illustrated by the following simulations. Using the conditions UBTLI = 500 µM, KBTLI = 10 µM, let MTDOSE range from 1 to 100 µmoles, the effects on AVGTL are illustrated in Figure 12A. The fraction of the dose in the liver is shown in Figure 12B. For a linear system, this fraction should remain constant. However, since the range of doses spans the range of binding capacity in the liver we see the influence of non-linear processes.
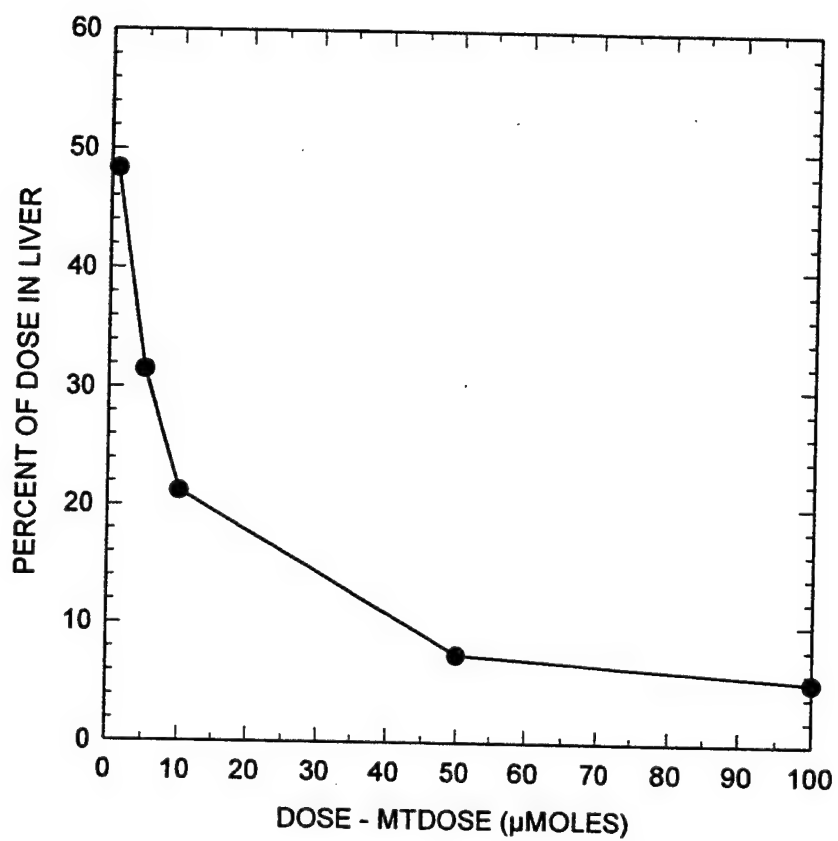
2.3.2 Binding Affinity

49

A
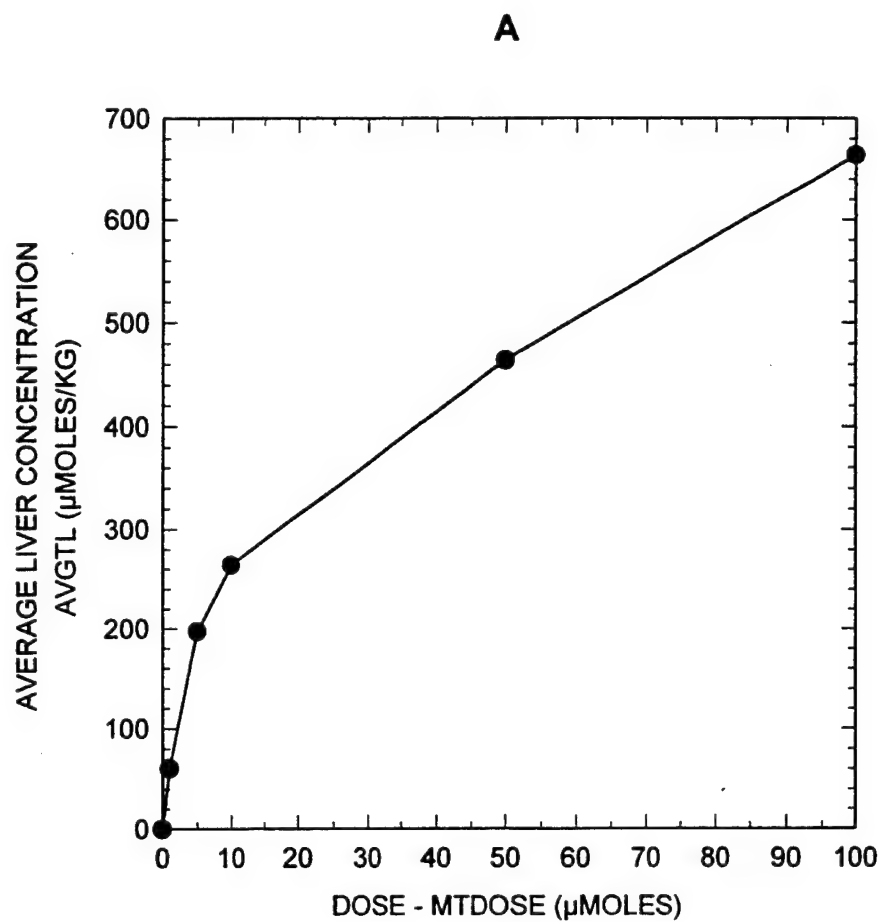
Figure 12: The Effect of Varying the Dose of Parent Chemical T on the Liver Concentration in the Presence of Intracellular Binding in the Isolated Perfused Rat Liver System.

(A) Average concentration of parent chemical in the liver at the end of the 2 h perfusion (AVGTL), and (B) percent of dose in the liver at the end of the perfusion.

B

Intracellular binding is controlled not only by the binding capacity of the intracellular ligand but also by the binding affinity. As an illustration, the effect of varying the binding affinity can be investigated for a given binding capacity. In this case the following conditions were used:

Membrane transport - simple diffusion, high permeability
Macromolecular binding - intracellular binding, vary KBTLI
Metabolism - none
Biliary excretion - none

The intracellular binding capacity was set at UBTLI = 1000 μM. Figure 13A illustrates the effect of binding affinity on the total (free) concentration of a chemical in the perfusion medium. As the binding affinity is increased, as measured by KBTLI decreasing from 100 μM to 0.1 μM, more chemical is sequestered in the liver as the bound complex, thus the concentration in the PM decreases Two interesting features of the kinetics are apparent in Figure 13A. First, the extraction of chemical from the PM is transport limited and second, the amount extracted reaches a maximum when the intracellular ligand is saturated. The saturation effect can be observed by looking at the intracellular bound concentration, DTLI(2), as illustrated in Figure 13B. As the affinity increases (KBTLI decreasing), the capacity of the intracellular ligand is saturated. At this point the remaining chemical will passively distribute as the free species. Comparing the free intracellular concentration, DTLI(1) (Figure 13C), with that in the PM, it is obvious that at equilibrium these two concentrations are equal as expected. Finally, the average liver concentration is shown in Figure 13D. The concentration in the liver is highly elevated relative to the PM. The partition coefficient ranges from 0.79 in the case of no binding to 24 in the case of strong binding (KBTLI = 0.1 μM). From these results it is clear that both capacity and affinity of intracellular binding will play an important role in the effect of protein binding on chemical kinetics.

2.4 EFFECT OF MACROMOLECULAR BINDING IN THE PERFUSION MEDIUM

The previous section discussed the effect of intracellular binding on the kinetics of chemicals in the IPRL system. In this section, the effect of protein binding in the PM will be explored. Since the only macromolecule available for binding in the PM is albumin, the following discussion will assume that there is only one binding site on bovine serum albumin for the chemical of interest. Thus, the binding capacity in the PM will be equal to the concentration of albumin, UBTP = 600 μM. The effect of varying the binding affinity of the chemical for albumin will be investigated in the presence of moderate binding in the intracellular space (UBTLI = 1000 μM, KBTLI = 10 μM). For the conditions

A
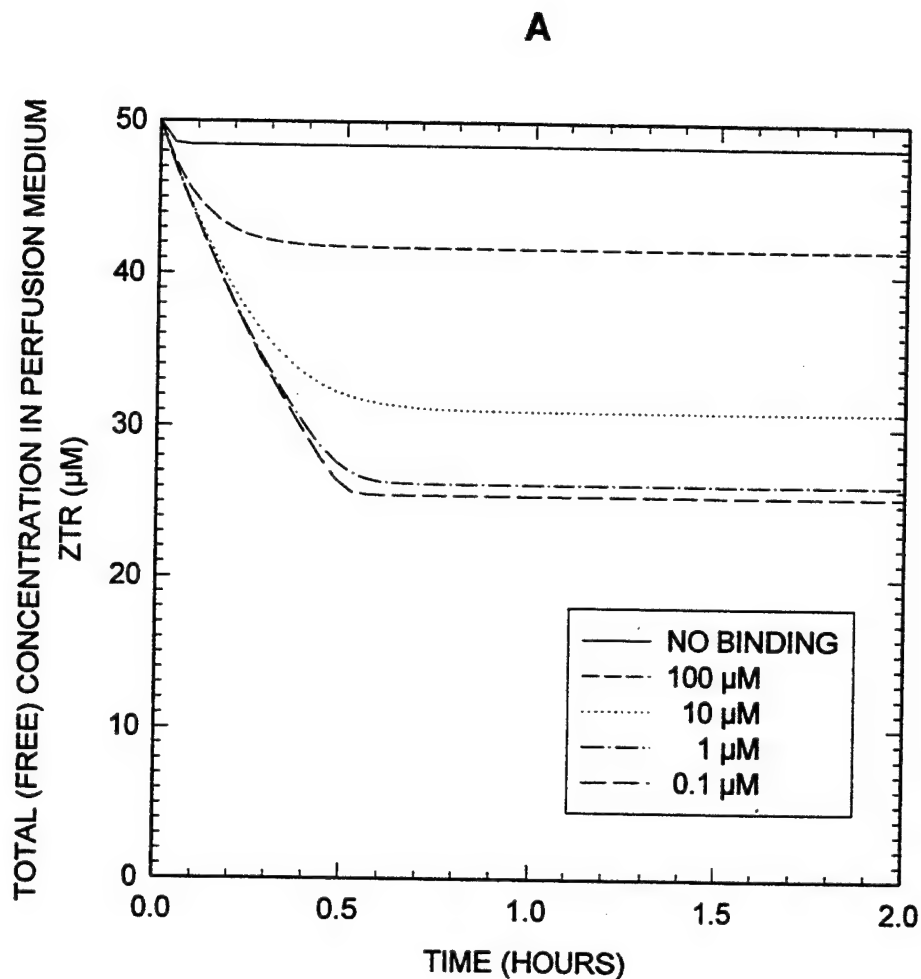
Figure 13: Effect of Binding Affinity of the Intracellular Ligand on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) bound concentration of the parent chemical in the intracellular water space (DTLI(2)), (C) free concentration of the parent chemical in the intracellular water space (DTLI(1)), and (D) average concentration of parent chemical in the liver (AVGTL). UBTLI = 10^3 µM, KBTLI varied from 100 to 0.1 µM

B

Figure axes: Y-axis "BOUND CONCENTRATION IN INTRACELLULAR SPACE DTLI(2) (µM)" with values 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000; X-axis "TIME (H)" with values 0.0, 0.5, 1.0, 1.5, 2.0.

Legend:
NO BINDING
100 µM
10 µM
1 µM
0.1 µM

C

FREE CONCENTRATION IN INTRACELLULAR SPACE DTLI(1) (µM) vs TIME (H)

Legend:
- NO BINDING
- 100 µM
- 10 µM
- 1 µM
- 0.1 µM

D

AVERAGE LIVER CONCENTRATION AVGTL (µM)

TIME (H)

Legend:
- NO BINDING
- 100 µM
- 10 µM
- 1 µM
- 0.1 µM
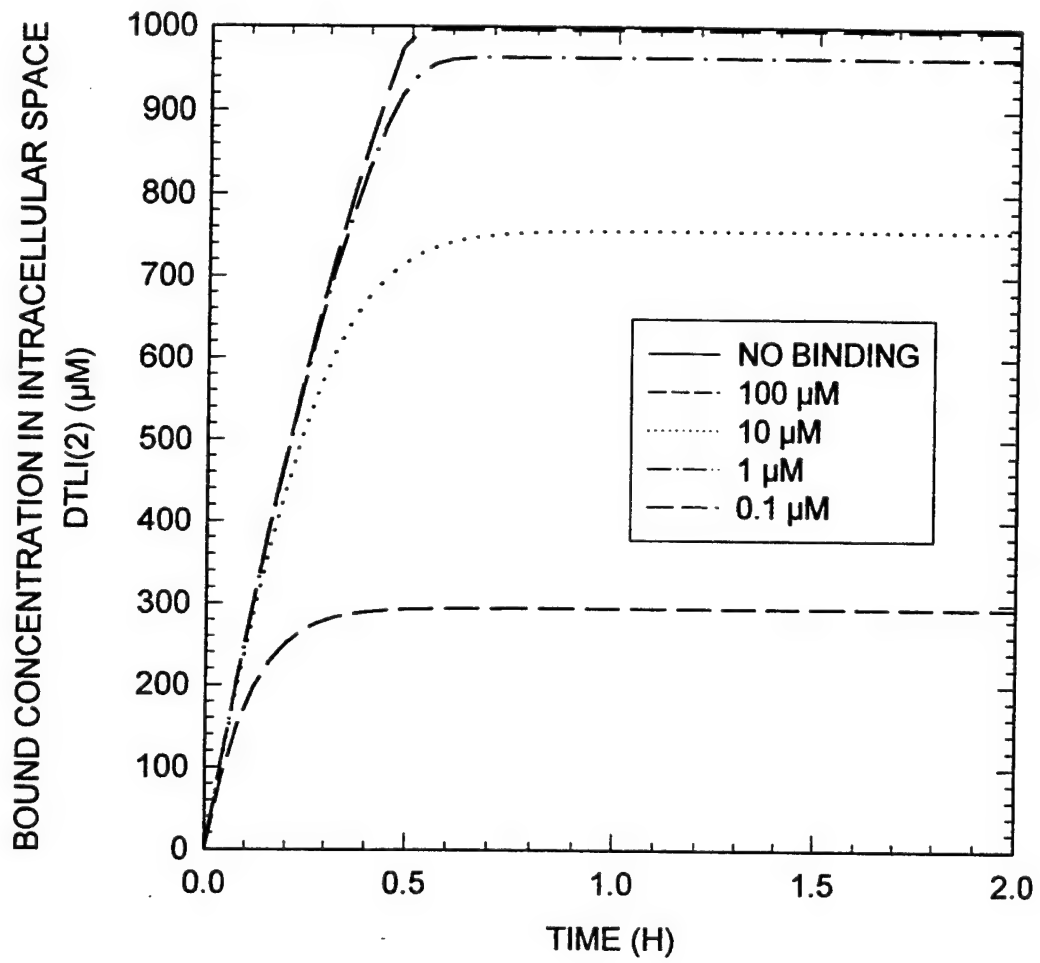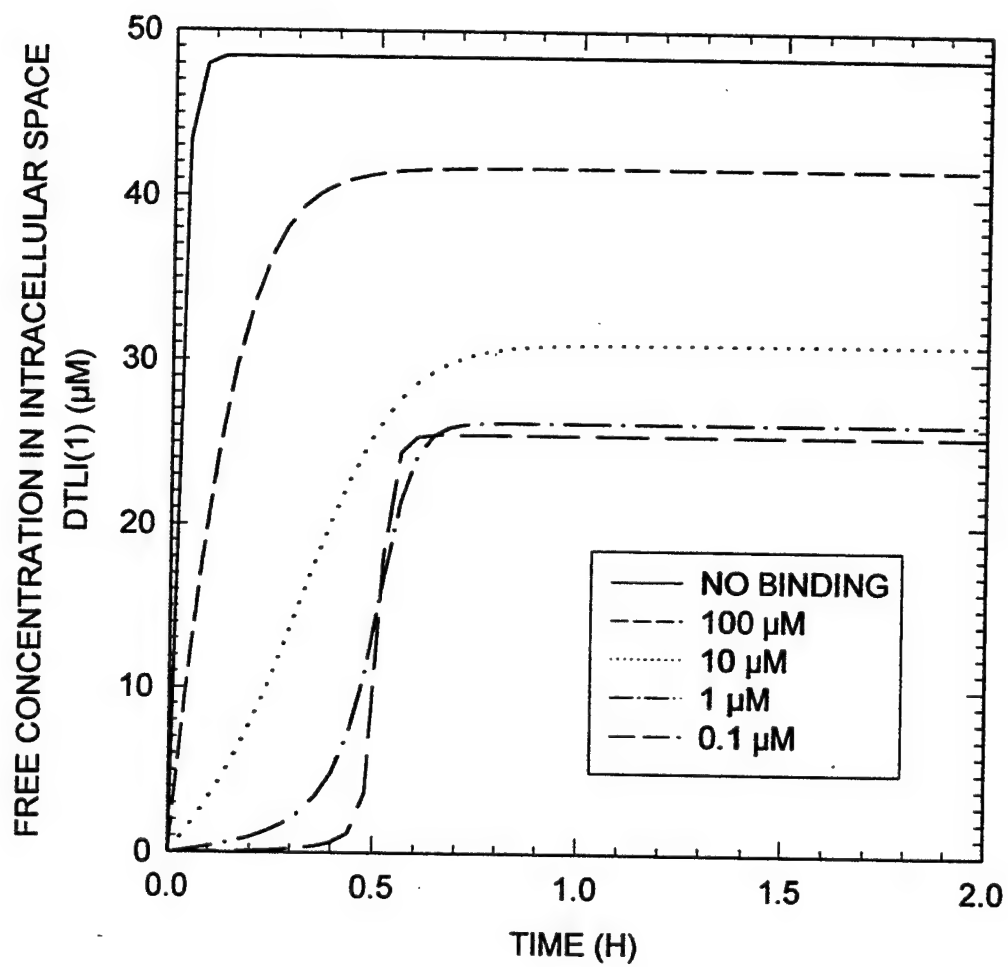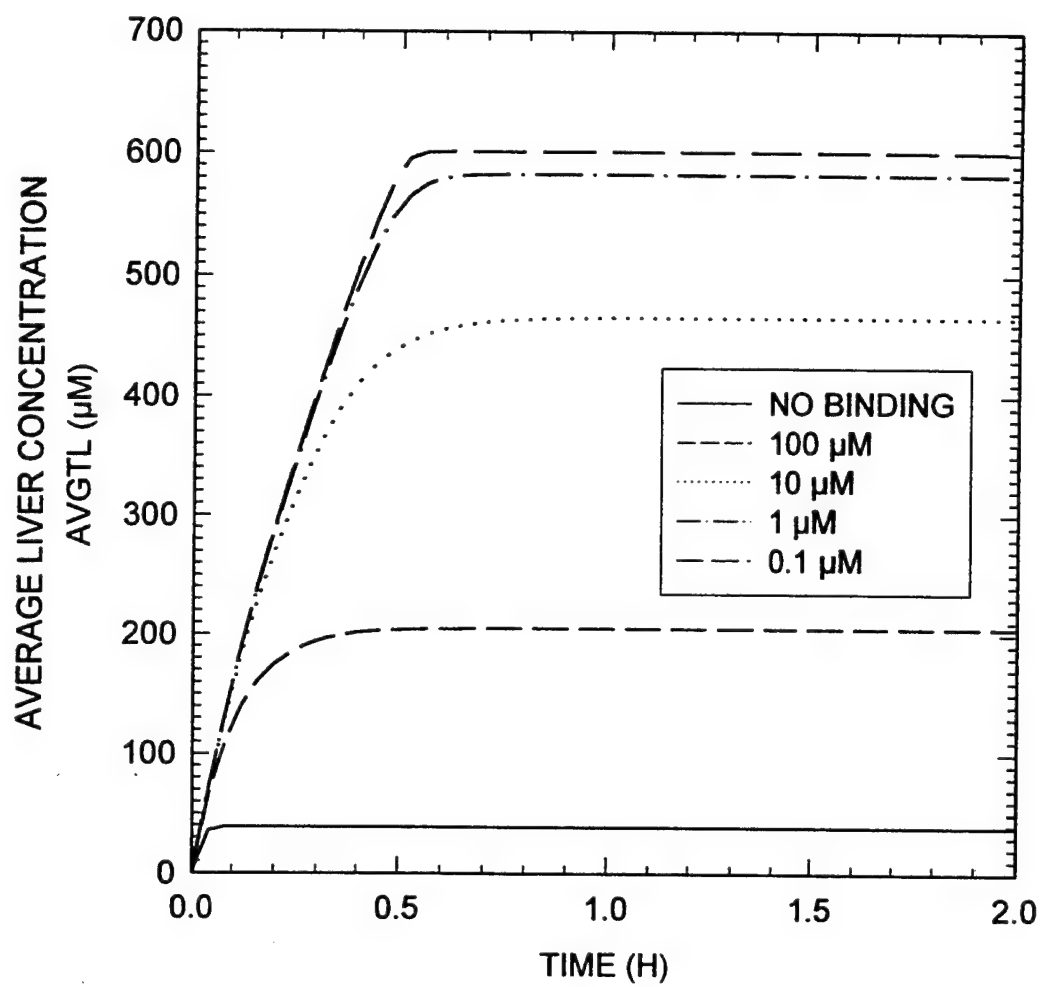
Membrane transport - simple diffusion, high permeability
Macromolecular binding - moderate intracellular binding, variable affinity of binding
    in the PM - vary KBTP
Metabolism - none
Biliary excretion - none

the concentration of the chemical in the PM is given in Figure 14A. With no albumin binding in the PM, the concentration in the PM decreases as the chemical is sequestered in the liver intracellular space due to intracellular binding. As albumin binding is turned on, less of the chemical is sequestered in the liver and more remains in the PM as the albumin bound complex, Figure 14B. In fact, at very high affinity of the chemical for albumin in the PM, most of the chemical is in the PM in the bound form. Thus, as the albumin-chemical affinity increases, albumin effectively competes with the intracellular ligand for binding of the chemical. With both albumin and intracellular binding the chemical, the free concentration of the chemical at equilibrium becomes very low, Figure 14C. Most of the chemical is either bound to albumin or the intracellular ligand. Figure 14D illustrates the depletion of the intracellular chemical-ligand complex with increasing albumin binding affinity, and this depletion is reflected in the decrease in average liver concentration, Figure 14E.

These simulations illustrate the influence of competition between binding ligands in the PM and in the intracellular space on chemical kinetics. The ultimate distribution is determined by the relative capacities and affinities of the competing ligands.

2.5 EFFECT OF METABOLISM

2.5.1 Basic Metabolism

Metabolism of the parent chemical in the liver intracellular space converts the IPRL system from a closed system to an open system with respect to the parent chemical. The presence of a metabolite means that the kinetic processes that have been discussed above for the parent chemical must be taken into consideration for the metabolite as well. To investigate the behavior of the parent chemical and the metabolite in the IPRL system in the presence of metabolism, the following conditions were considered

Membrane transport - parent chemical - simple diffusion, high permeability,
    metabolite - simple diffusion, moderate permeability
Macromolecular binding - no binding of parent chemical or metabolite
Metabolism - variable V_{MAX}, moderate K_M
Biliary excretion - none

A
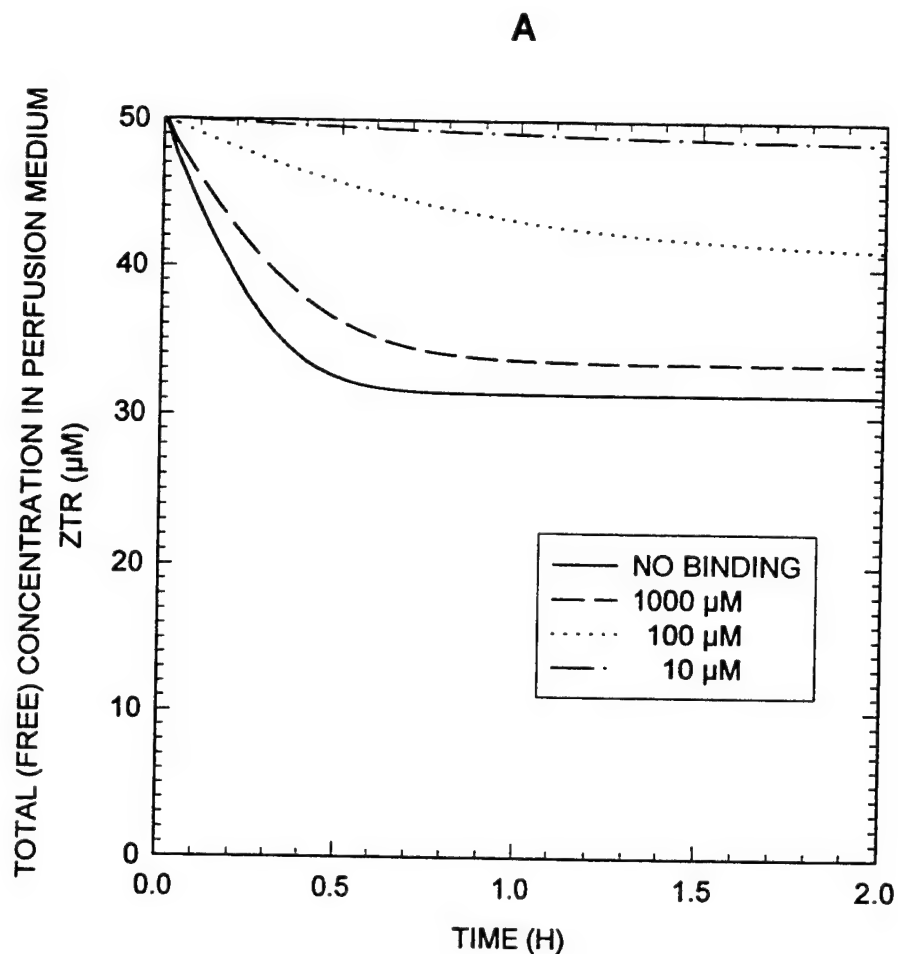
Figure 14: Effect of Albumin Binding in the Perfusion Medium on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

(A) Total concentration of the parent chemical in the PM (ZTR), (B) bound concentration in the PM (DTR(2)), (C) free concentration of the parent chemical in the intracellular water space (DTLI(1)), (D) bound concentration in the intracellular space (DTLI(2)), and (E) average concentration of parent chemical in the liver (AVGTL). The condition for no binding in the PM is given for reference. KBTP is varied from 10^3 to 10 μM.

B

C

D

E

Legend:
- NO BINDING
- 1000 μM
- 100 μM
- 10 μM

Y-axis: AVERAGE LIVER CONCENTRATION AVGTL (μMOLE/KG)

X-axis: TIME (H)
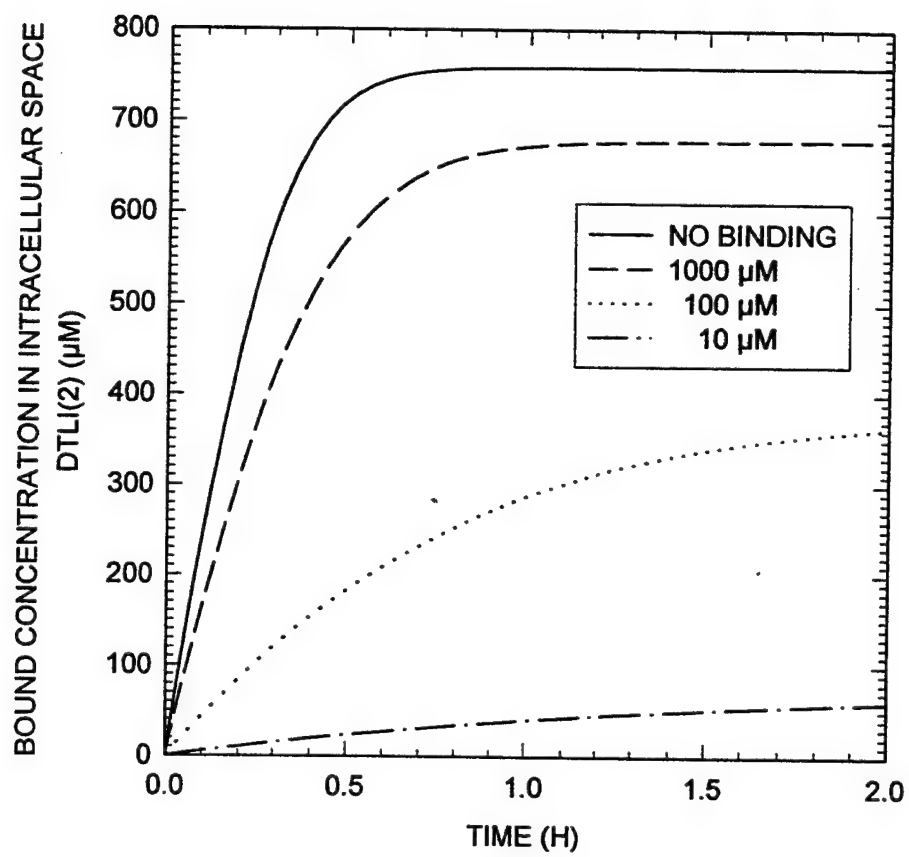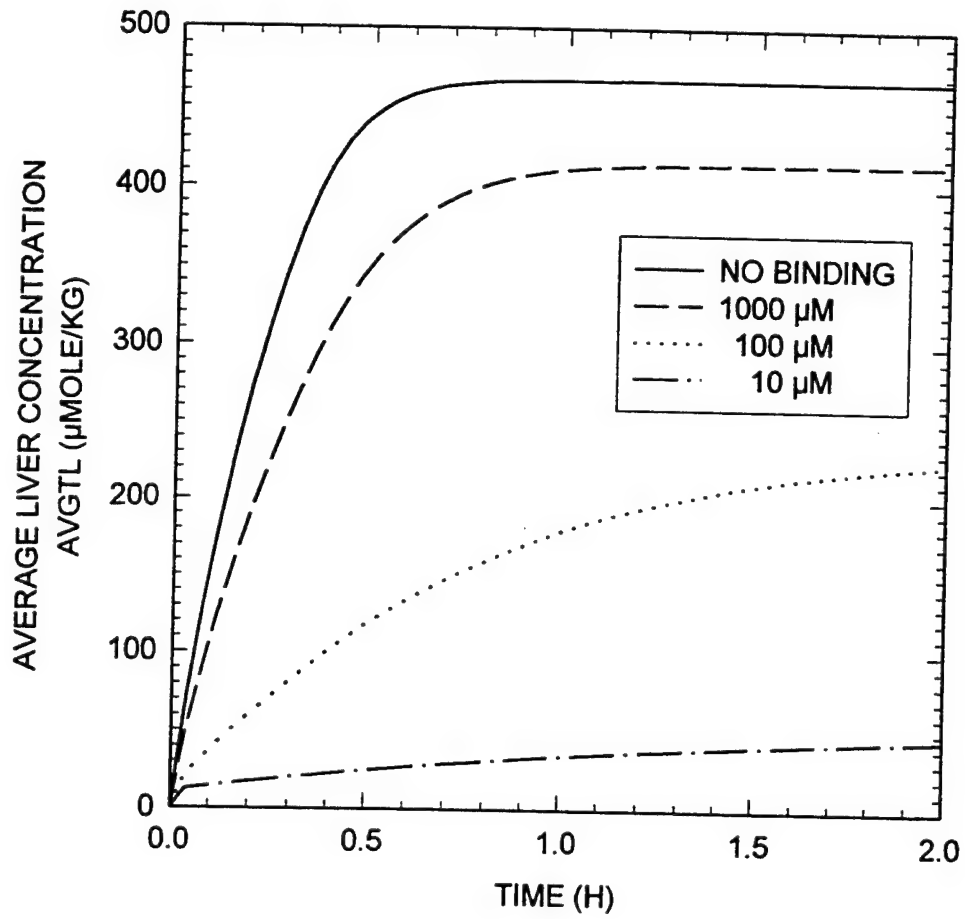
Figure 15A illustrates the disappearance of the parent chemical from the perfusion medium as the maximum rate of metabolism, SVTMAX {μmoles/h*Kg}, is increased from 0 to 10,000 μmoles/h*Kg of liver. At the highest rate, almost all of the parent chemical is metabolized during the 2 h perfusion. The loss of the parent chemical is mirrored by the appearance of metabolite in the PM, Figure 15B. Increasing the V_{MAX} beyond this level will have little additional effect on elimination of the parent chemical since delivery of the chemical to the site of metabolic activity is rate limiting, not the enzymatic activity. This can be seen in Figure 15C, where the free parent concentration in the intracellular compartment is plotted. At the highest metabolic rate, the chemical is metabolized almost as fast as it enters the intracellular compartment, i.e., the free concentration of the parent chemical is almost zero. The average liver concentration of the parent chemical is significantly reduced by metabolism, Figure 15D, while that of the metabolite increases, Figure 15E.

The presence of metabolism in the liver has a significant effect on the kinetics of the chemical in the IPRL system. The fact that the system becomes open with respect to the parent chemical results in the ultimate disappearance of the parent chemical and conversion to the metabolite.

2.5.2 Interaction Between Metabolism and Membrane Transport

To investigate the effect of membrane transport on metabolism of the parent chemical within the intracellular space, the model was exercised with a moderate level of metabolism and a variable diffusional rate. The situation simulated was
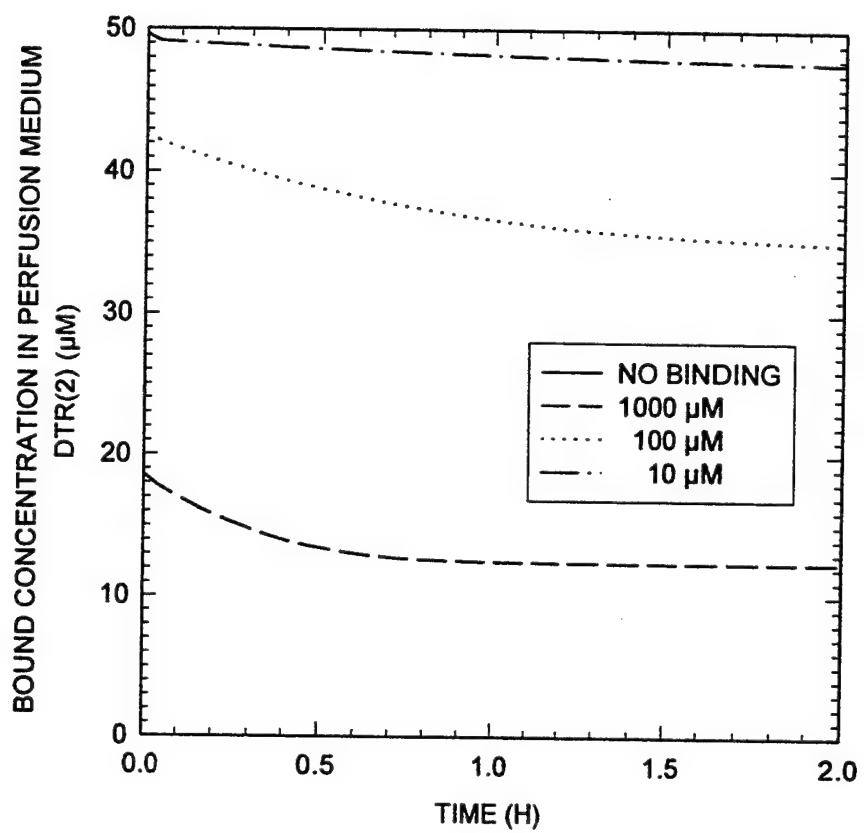
Membrane transport - parent chemical - simple diffusion, variable permeability; metabolite - simple diffusion, moderate permeability
Macromolecular binding - no binding of parent or metabolite
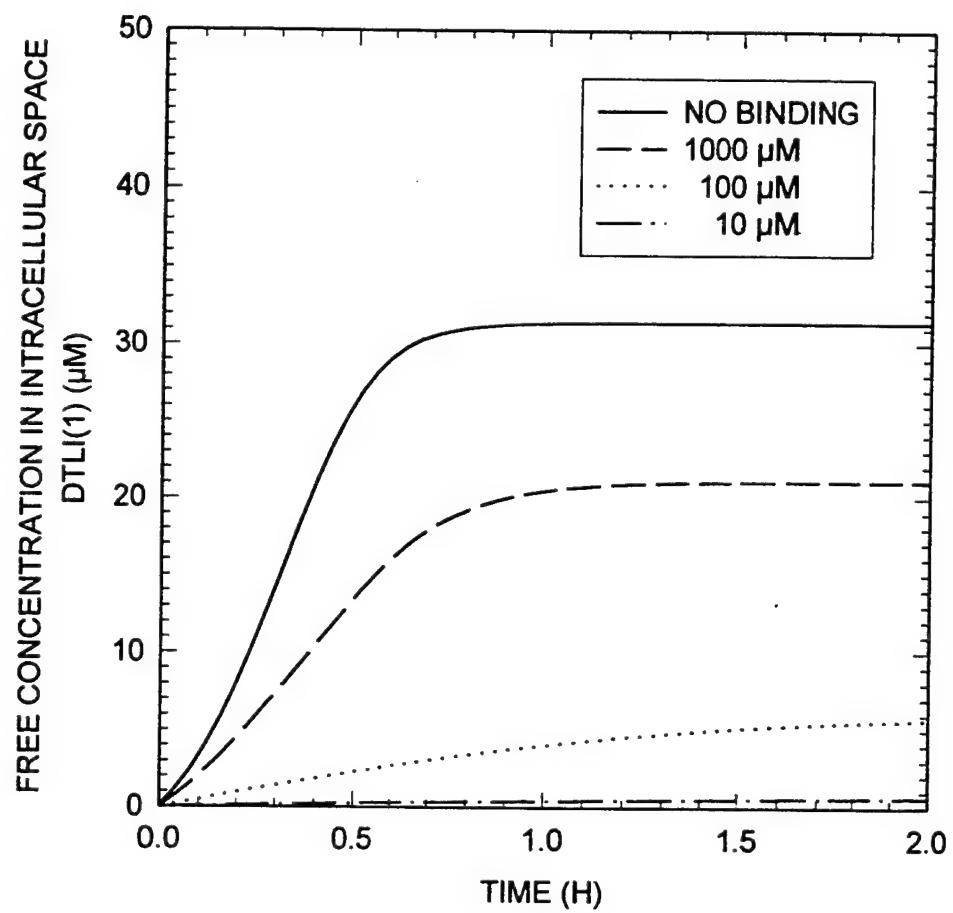Metabolism - moderate V_{MAX} and K_M
Biliary excretion - none

The chemical dose was 10 μmoles and the metabolic parameters were SVTMAX = 10^3 μmoles/h*kg and KMTLI = 10 μM. The behavior of the parent chemical in the PM is illustrated in Figure 16A. When the PA product was zero, no chemical entered the intracellular space and consequently no chemical was metabolized. As the permeability is increased slightly, the rate of metabolism increases, parent chemical is cleared from the PM and metabolite appears in the PM (Figure 16B). As the PA product increases to high levels, the parent chemical rapidly equilibrates across the sinusoidal-intracellular interface and the free concentration of the parent chemical in the intracellular space increases to levels comparable to that in the PM (Figure 16C). When the free intracellular concentration of the parent chemical (substrate) becomes large, the metabolic pathway becomes saturated (free concentration of substrate is much greater than K_M) and the rate

63

A
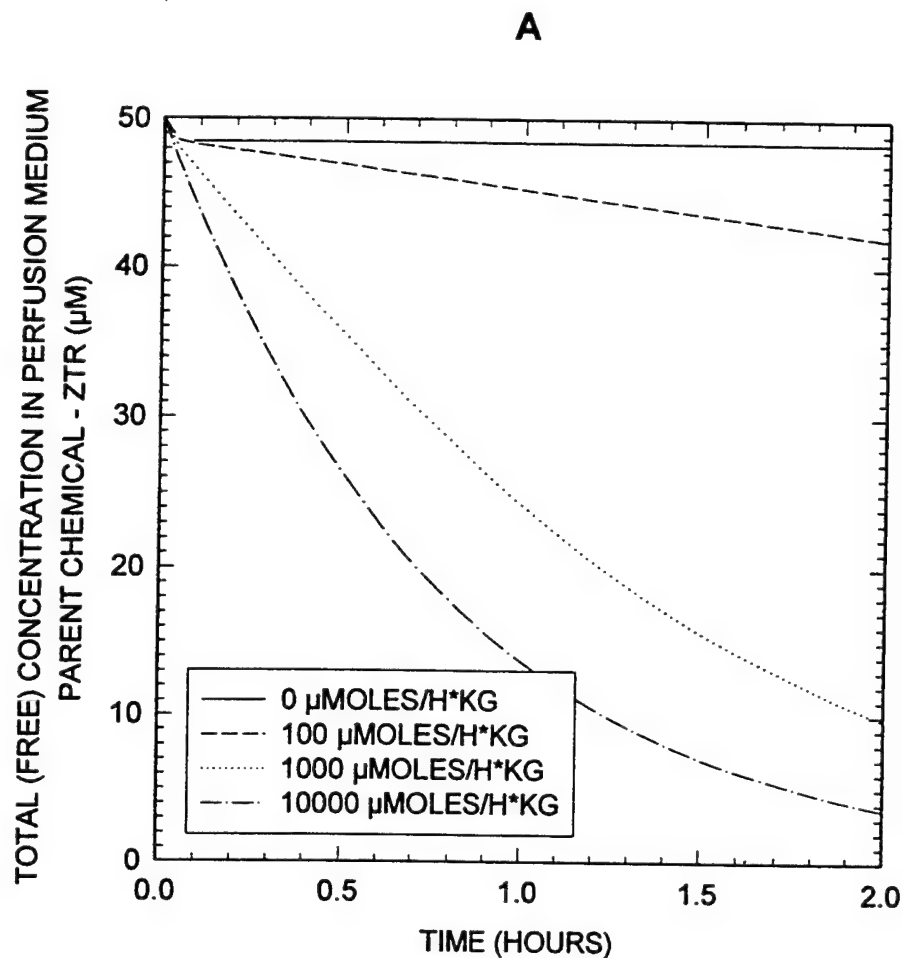
Figure 15: Effect of Metabolism on the Kinetics of the Parent Chemical T and the Metabolite M in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) total (free) concentration of the metabolite in the PM (ZMR), (C) total (free) concentration of the parent chemical in the intracellular water space ((DTLI(1)), (D) average concentration of parent chemical in the liver (AVGTL), (E) free concentration of metabolite in the intracellular space (DMLI(1)). SVTMAX varied from 0 to 10^4 µmoles/h∗Kg.

B

TOTAL (FREE) CONCENTRATION IN PERFUSION MEDIUM METABOLITE - ZMR (μM)

Legend:
- 0 μMOLES/H*KG
- 100 μMOLES/H*KG
- 1000 μMOLES/H*KG
- 10000 μMOLES/H*KG

TIME (HOURS)

C

D

E

Figure showing free concentration in intracellular space metabolite - DMLI(1) (µM) versus time (H). X-axis: TIME (H) from 0.0 to 2.0. Y-axis: FREE CONCENTRATION IN INTRACELLULAR SPACE METABOLITE - DMLI(1) (µM) from 0 to 300. Legend: 0 µMOLES/H*KG; 100 µMOLES/H*KG; 1000 µMOLES/H*KG; 10000 µMOLES/H*KG.

A

Figure 16: **Effect of the Interaction of Membrane Transport and Metabolism on the Kinetics of the Parent Chemical T and the Metabolite M in the Isolated Perfused Rat Liver System.**

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) total (free) concentration of the metabolite M in the PM (ZMR), (C) total (free) concentration of the parent chemical in the intracellular water space (DTLI(1)), (D) average concentration of parent chemical in the liver (AVGTL), (E) average concentration of the metabolite in the liver (AVGML). ALSI*PTLSI = ALSI*PTLIS is varied from 0 to 10^2 cm³/sec.

69

B

Figure showing TOTAL (FREE) CONCENTRATION IN PERFUSION MEDIUM METABOLITE - ZMR (μM) versus TIME (HOURS), with curves for:
- 0 CM^3/SEC
- 0.1 CM^3/SEC
- 1 CM^3/SEC
- 10 CM^3/SEC
- 100 CM^3/SEC

C

FREE CONCENTRATION IN INTRACELLULAR SPACE PARENT CHEMICAL - DTLI(1) (μM)

TIME (HOURS)

Legend:
- 0 CM^3/SEC
- 0.1 CM^3/SEC
- 1 CM^3/SEC
- 10 CM^3/SEC
- 100 CM^3/SEC

D

AVERAGE LIVER CONCENTRATION
PARENT CHEMICAL - AVGTL (μMOLES/KG)

TIME (HOURS)

Legend:
- 0 CM^3/SEC
- 0.1 CM^3/SEC
- 1 CM^3/SEC
- 10 CM^3/SEC
- 100 CM^3/SEC

E

of metabolism becomes zero order. The average concentrations of the parent chemical and metabolite in the liver are shown in Figures 16D and 16E, respectively. The average liver concentration of the parent chemical increases at early time points with increasing diffusional permeability, but later decreases as parent chemical is metabolized, ultimately decreasing to zero as the parent is completely converted to metabolite. At PA products below about 100 cm^3/sec, metabolism is limited by the diffusional barrier. These simulations emphasize the point that interactions between delivery of the chemical to the metabolic site and metabolic elimination can be important and require independent experimental measurements to resolve the combined effects.

2.5.3 Interaction Between Metabolism and Binding in the Perfusion Medium

Binding of the parent chemical to albumin in the perfusion medium will reduce the availability of the parent chemical (substrate) at the site of intracellular metabolism. The effects can be demonstrated under the following conditions:

> Membrane transport - parent chemical - simple diffusion, high permeability;
> metabolite - simple diffusion, moderate permeability
> Macromolecular binding - parent chemical - variable binding affinity in PM, no
> intracellular binding; no binding of metabolite
> Metabolism - moderate V_{MAX} and K_M
> Biliary excretion - none

Under these conditions, metabolism of the parent chemical will take place rapidly in the absence of protein binding, 80% of the parent chemical is eliminated from the PM during the 2 h perfusion (Figure 17A). As the binding affinity of albumin in the PM increases, the rate of metabolism decreases to the point where metabolism is experimentally non-measurable at very high affinity. The basis for this effect is obvious when the intracellular free concentration of the parent chemical is observed (Figure 17B). When binding in the PM becomes sufficiently great, the availability of the chemical to the metabolizing enzyme is reduced to practically zero. Thus, apparent metabolism ceases and little metabolite is found in the PM (Figure 17C). This result emphasizes the fact that protein binding in the PM can have a major impact on the apparent metabolic rate in tissues.

2.5.4 Interaction Between Metabolism and Intracellular Binding

Intracellular binding will also affect the rate of metabolism of a chemical, but it will have different effects on the kinetics of the parent chemical in the system than was observed in the case of binding in the PM. Figure 18A illustrates the effects of intracellular binding and metabolism on the kinetics of the chemical in the PM using the conditions

> Membrane transport - parent chemical - simple diffusion, high permeability;
> metabolite - simple diffusion, moderate permeability
> Macromolecular binding - parent chemical no binding in PM, variable intracellualr
> binding; no binding of the metabolite

74

A
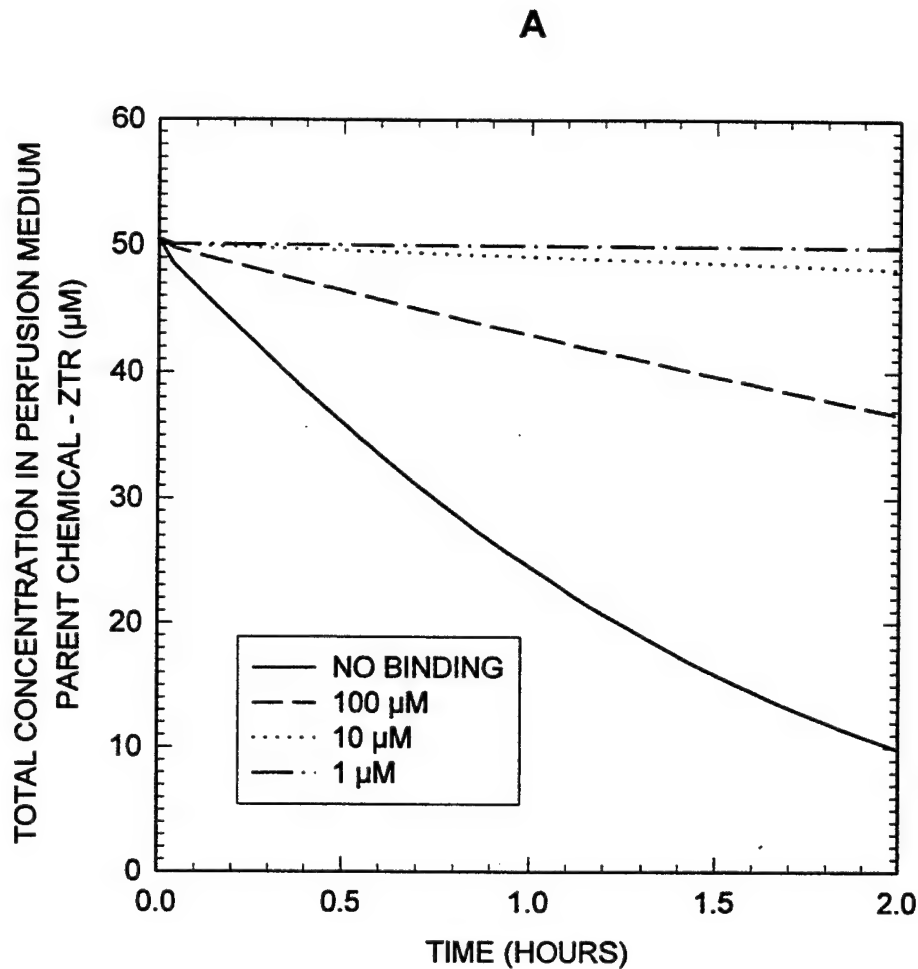
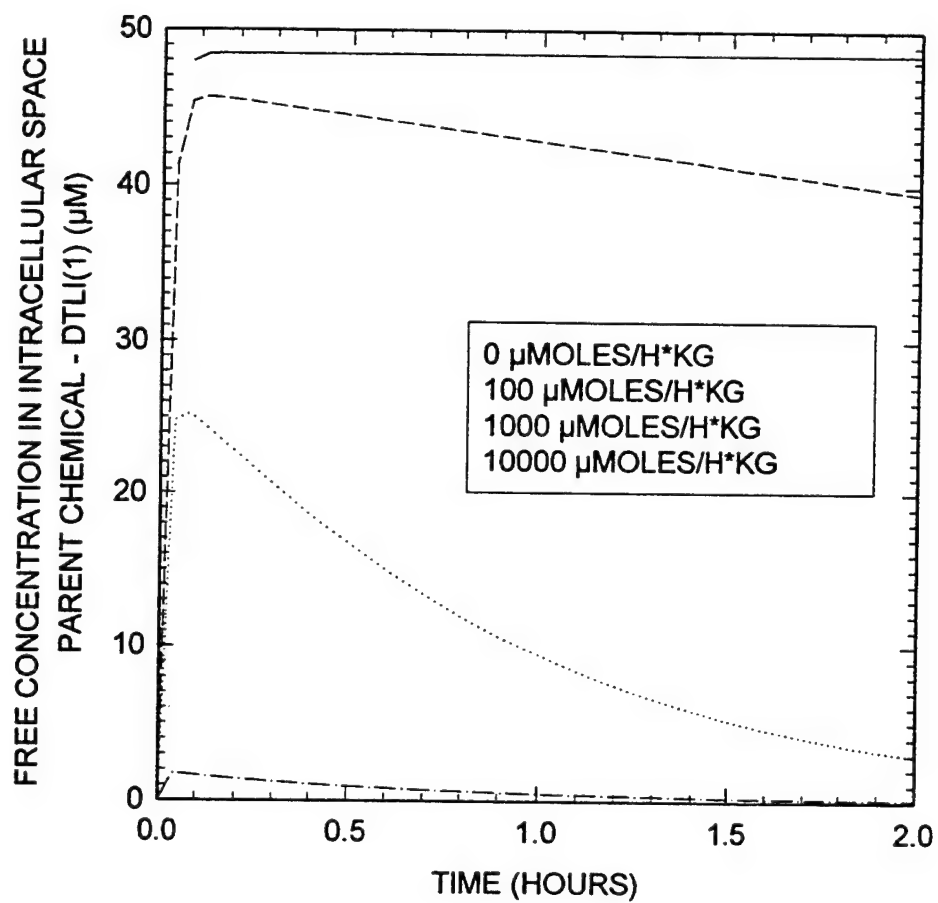Figure 17: Effect of Interaction of Binding of the Parent Chemical in the Perfusion Medium and Metabolism on the Kinetics of the Parent Chemical T and Metabolite M in the Isolated Perfused Rat Liver System.

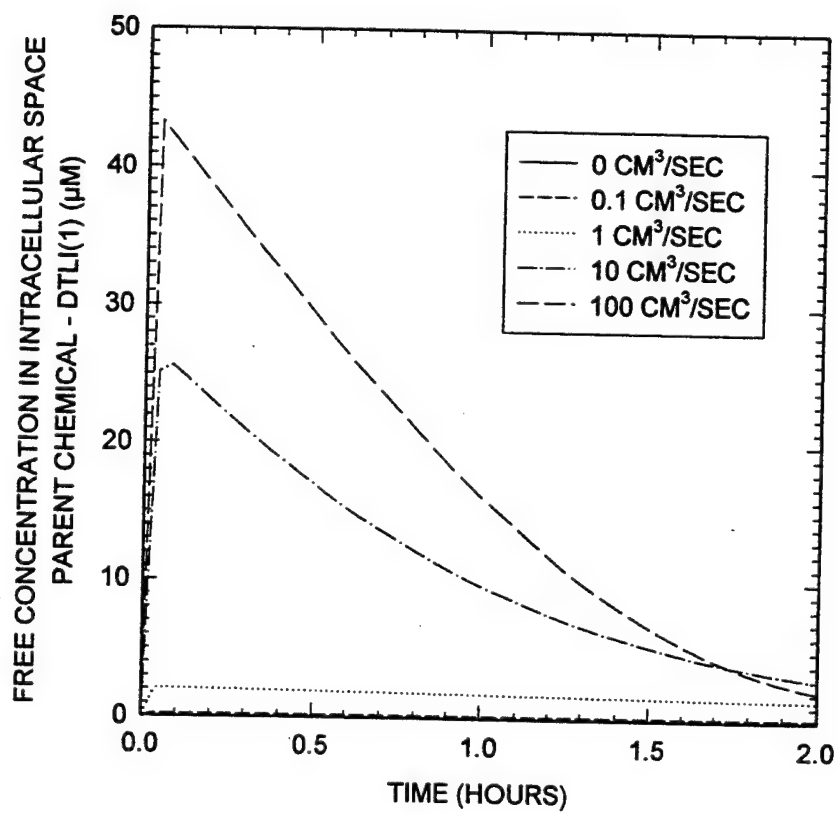(A) Total concentration of the parent chemical in the PM (ZTR), (B) free concentration of the parent chemical in the intracellular water space (DTLI(1)), and (C) total (free) concentration of the metabolite in the PM (ZMR). UBTP is varied from 0 to 100 μM.

75

B

C

TOTAL (FREE) CONCENTRATION IN PERFUSION MEDIUM METABOLITE - ZMR (μM)

Legend:
- NO BINDING
- 100 μM
- 10 μM
- 1 μM

TIME (HOURS)
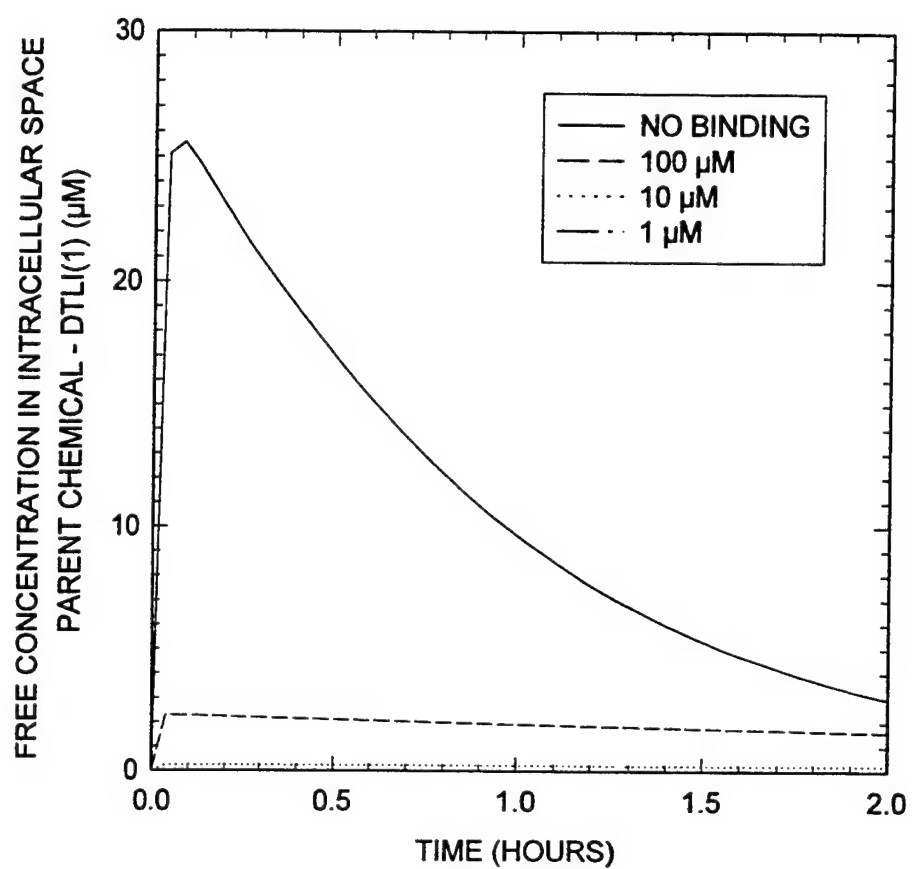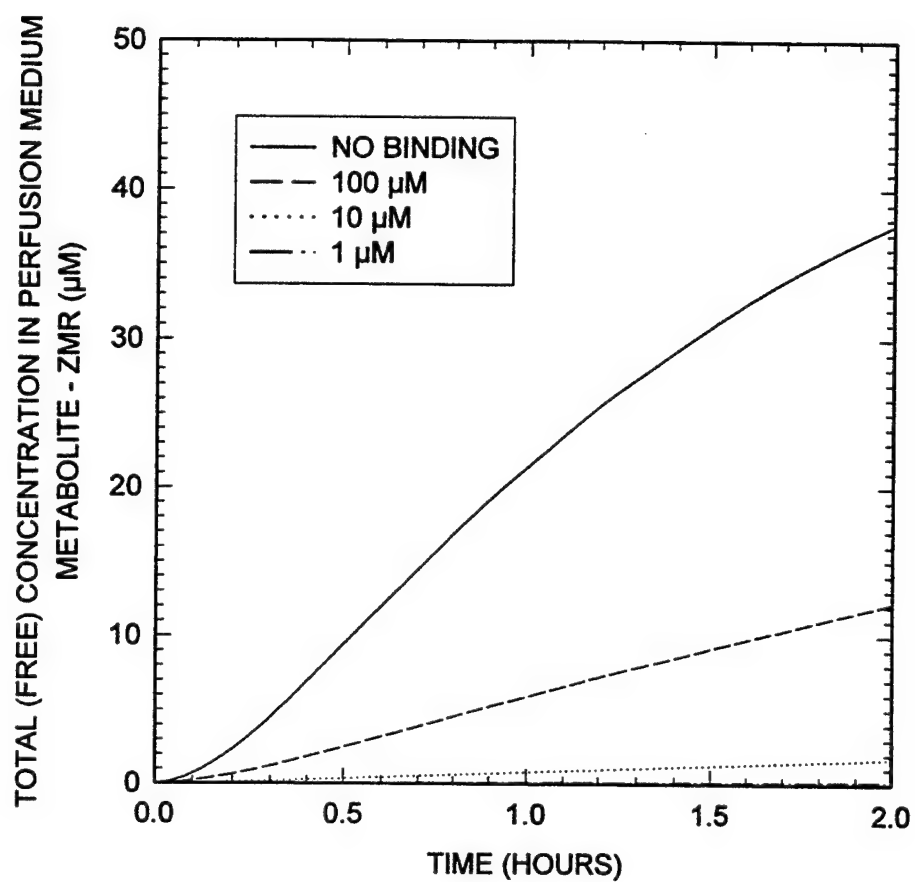
Metabolism - moderate V_{MAX}, moderate K_M
Biliary excretion - none

In this case, as intracellular binding increases, the concentration of the chemical in the PM decreases more rapidly than the rate observed with metabolism alone. A more rapid elimination of the parent chemical from the PM usually implies increased metabolism. However, in this case, a portion of the parent chemical is not being converted to the metabolite but is being sequestered by the intracellular ligand. In fact, the amount of metabolite in the PM decreases, Figure 18B, as a result of the decrease in available substrate in the intracellular space (Figure 18C). Ultimately, all of the chemical will be converted to metabolite, but in the presence of protein binding it will take much longer to complete the reaction. Protein binding will significantly affect the apparent V_{MAX} for an enzymatic process. The average liver concentration of the parent chemical will increase with increasing intracellular binding, Figure 18D, but the average liver concentration of the metabolite will decrease, Figure 18E. Interactions between binding and metabolism can lead to counter-intuitive behaviors of the parent chemical and metabolite in the system.

2.6 EFFECT OF BILIARY EXCRETION

2.6.1 Linear Biliary Excretion

Chemicals can be transported into the bile by various mechanisms. If the transport process is linear, the rate of elimination via the biliary route can be described by a single transport parameter that relates the rate of transport to the concentration of the chemical in the available intracellular pool, presumably the free chemical pool. Figure 19A shows the effect of linear biliary elimination on the concentration of the parent chemical in the PM using the following conditions:

Membrane transport - simple diffusion, high permeability
Macromolecular binding - no binding
Metabolism - no metabolism
Biliary excretion - linear biliary excretion

The proportionality constant, STTB1 {L/h*Kg}, is the rate constant for transport of the chemical into bile per unit available concentration in the intracellular space per Kg of liver weight. This is a specific rate constant, i.e., normalized per weight of liver, and must be scaled by liver size for a specific experiment. As the rate constant increases, the concentration in the PM decreases more rapidly, Figure 19A. At very high biliary excretion rates, the elimination of the parent chemical from the PM is transport limited at the intracellular -cannilicular barrier. The free concentration in the liver intracellular space, Figure 19B, is depressed as the elimination rate increases, so that at the highest rate, the

A
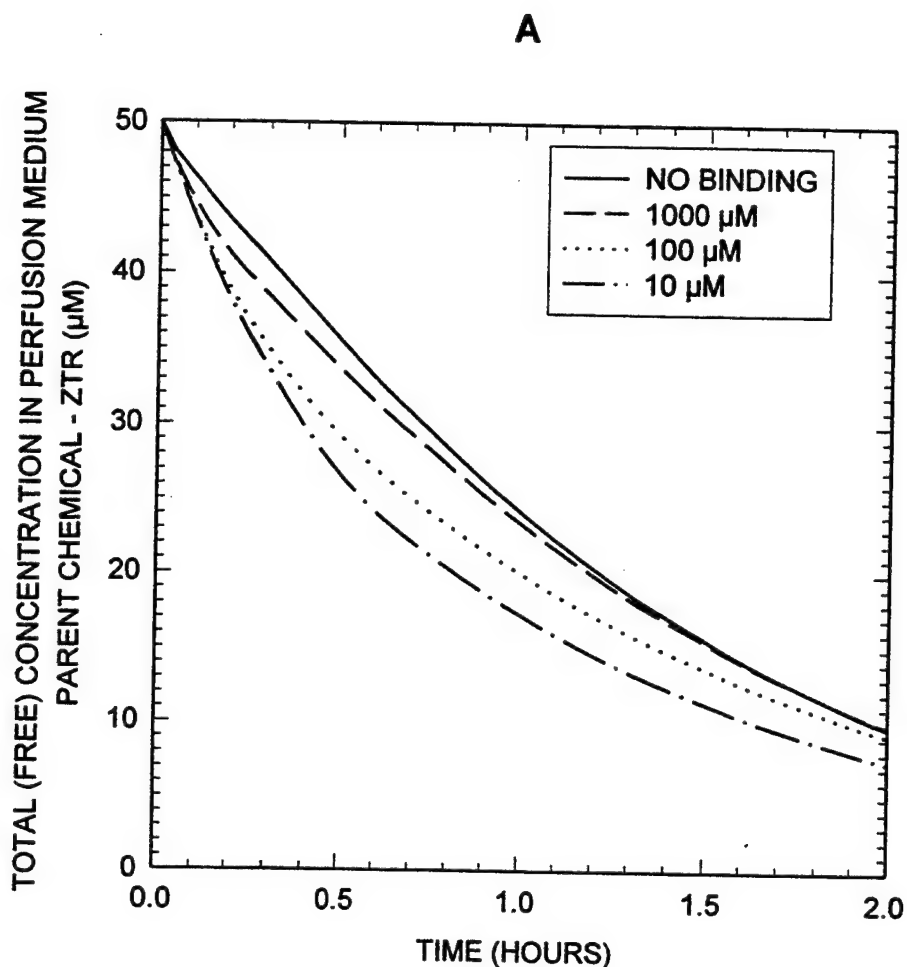
Figure 18: Effect of Interactions Between Intracellular Binding and Metabolism on the Kinetics of the Parent Chemical T and the Metabolite M in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) total (free) concentration of the metabolite in the PM (ZMR), (C) free concentration of the parent chemical in the intracellular water space (DTLI(1)), (D) average concentration of parent chemical in the liver (AVGTL), and (E) average concentration of the metabolite in the liver (AVGML). UBTLI varied from 0 to 10^3 µM.

B

C

The figure is a line plot. The y-axis is labeled "FREE CONCENTRATION IN INTRACELLULAR SPACE PARENT CHEMICAL - DTLI(1) (µM)" ranging from 0 to 50. The x-axis is labeled "TIME (HOURS)" ranging from 0.0 to 2.0. A legend box contains:

NO BINDING
1000 µM
100 µM
10 µM

D

Figure D. Average liver concentration of parent chemical (AVGTL, μM) versus time (hours). Curves shown for NO BINDING, 1000 μM, 100 μM, and 10 μM.

E

AVERAGE LIVER CONCENTRATION
METBOLITE - AVGML (µM)

TIME (HOURS)

NO BINDING
1000 µM
100 µM
10 µM

83

A

Figure 19: Effect of Linear Biliary Elimination on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) total (free) concentration of the parent chemical in the intracellular water space (DTLI(1)), (B) average concentration of parent chemical in the liver (AVGTL), and (D) cumulative biliary excretion of parent chemical (MTB).

B

C

AVERAGE LIVER CONCENTRATION AVGTL (µMOLES/KG) vs TIME (H)

Legend:
- 0 L/H*KG
- 1 L/H*KG
- 10 L/H*KG
- 100 L/H*KG
- 1000 L/H*KG

D
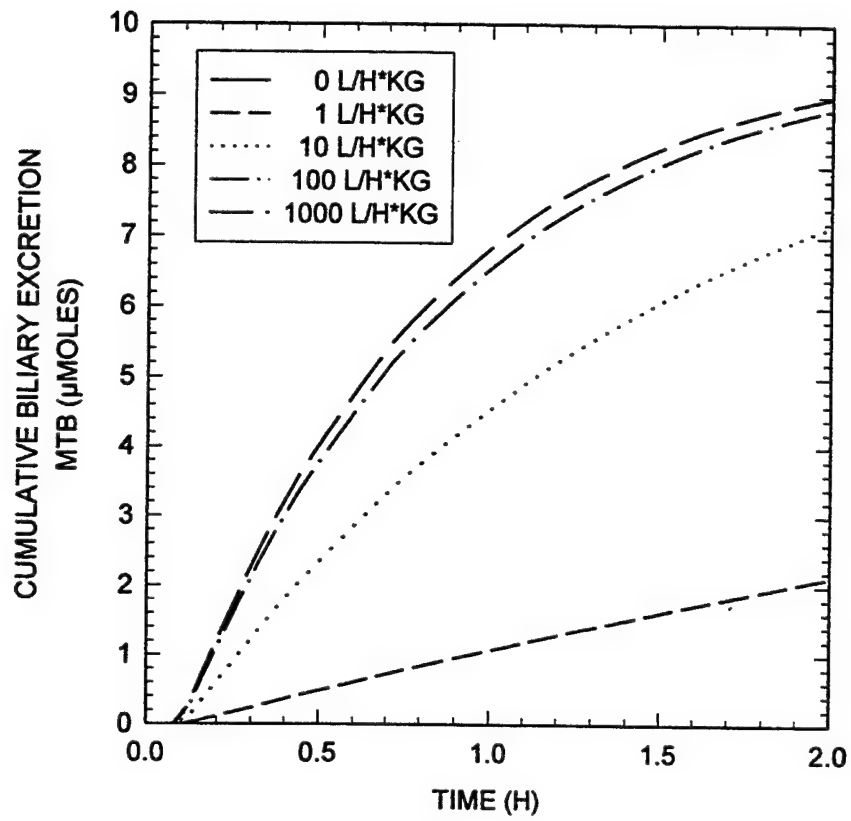
chemical is eliminated in the bile almost as fast as it enters the intracellular space. The average liver concentration, Figure 19C, reflects the rapid elimination of the chemical from the liver into the bile and the cumulative elimination in the bile, Figure 19D, increases more rapidly as STTB1 increases. Note the time delay in the appearance of the parent chemical in the bile is due to transport of the bile through the collection cannula. In all cases, the total chemical added to the system will be eliminated in the bile ultimately, it just takes longer when the rate constant is small.

2.6.2 Saturable Biliary Excretion

There are many chemicals that are excreted into the bile by saturable transport mechanisms. If we assume that biliary transport of the chemical of interest utilizes a carrier mediated process, then the simplest approach is to assume a Michaelis-Menton type relationship between the rate of chemical transport into the bile and the free concentration of the chemical in the liver intracellular space. The overall effect of the normalized maximum rate of transport, STTB2 {μmoles/h*Kg), on the kinetics of the chemical for a given fixed dose is similar to that observed for the linear case - compare Figures 20A-D to the corresponding graphs for the linear elimination case, Figures 19A-D. One way to investigate the non-linear behavior in the case of saturable transport processes is to investigate the effect of varying the dose on the observed kinetics. This is illustrated in Figure 21. In Figure 21A, the effect of varying the dose on the kinetics of the chemical in the PM are illustrated. A log scale is used to emphasize the change in the slope of the elimination curves. As the dose increases from 1 to 100 μmoles, the slope decreases indicating that the apparent elimination rate constant is dose dependent. This would be one sign of non-linear behavior. A similar pattern is displayed by the free intracellular concentration of the parent chemical (Figure 21B). Saturation of the biliary elimination pathway is also apparent in the cumulative biliary excretion (Figure 21C). At the higher doses, the biliary elimination pathway is saturated and the rate of excretion into the bile, as indicated by the slope of the cumulative biliary excretion curve, reaches a maximum controlled by STTB2.

These simulations give some insight into the influence of biliary excretion on the kinetics of chemicals in the IPRL system.

A
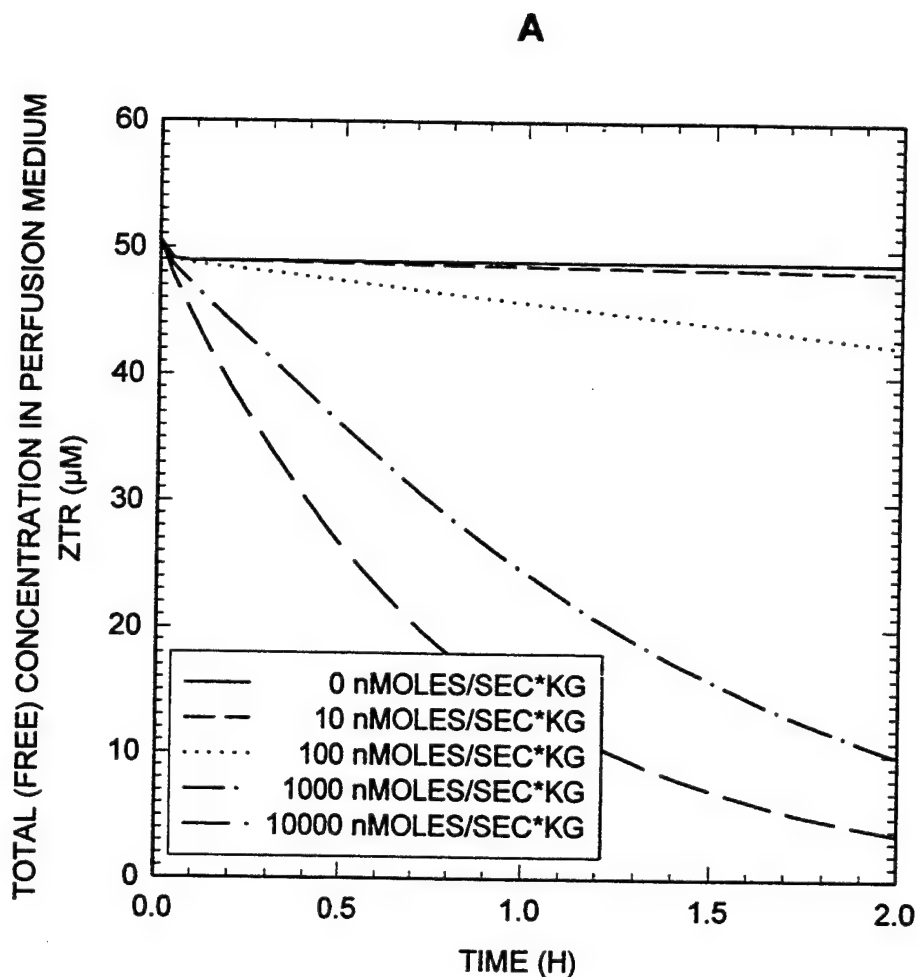
Figure 20: Effect of Saturable Biliary Elimination on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) total (free) concentration of the parent chemical in the intracellular space (DTLI(1)), (C) average concentration of parent chemical in the liver (AVGTL), (D) cumulative biliary excretion of parent chemical (MTB).

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B

TOTAL (FREE) CONCENTRATION IN INTRACELLULAR SPACE DTLI(1) (μM) vs TIME (H)

Legend:
- 0 nMOLES/SEC*KG
- 10 nMOLES/SEC*KG
- 100 nMOLES/SEC*KG
- 1000 nMOLES/SEC*KG
- 10000 nMOLES/SEC*KG

C

AVERAGE LIVER CONCENTRATION
AVGTL (µMOLES/KG)

TIME (H)

Legend:
- 0 nMOLES/SEC*KG
- 10 nMOLES/SEC*KG
- 100 nMOLES/SEC*KG
- 1000 nMOLES/SEC*KG
- 10000 nMOLES/SEC*KG
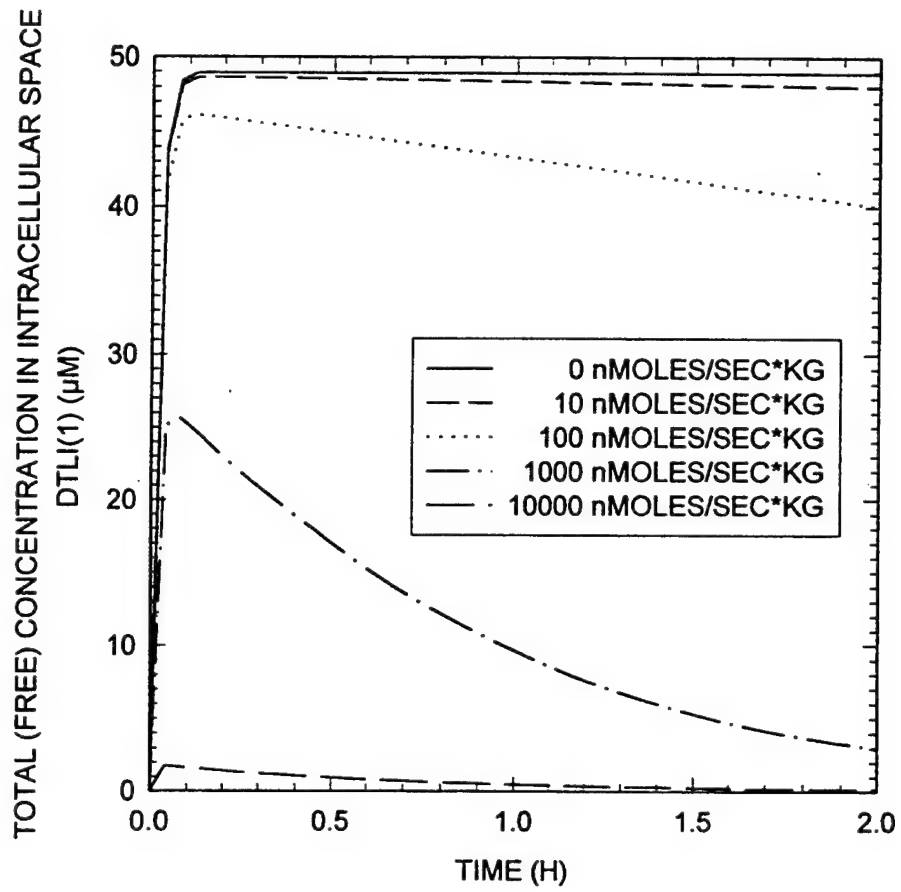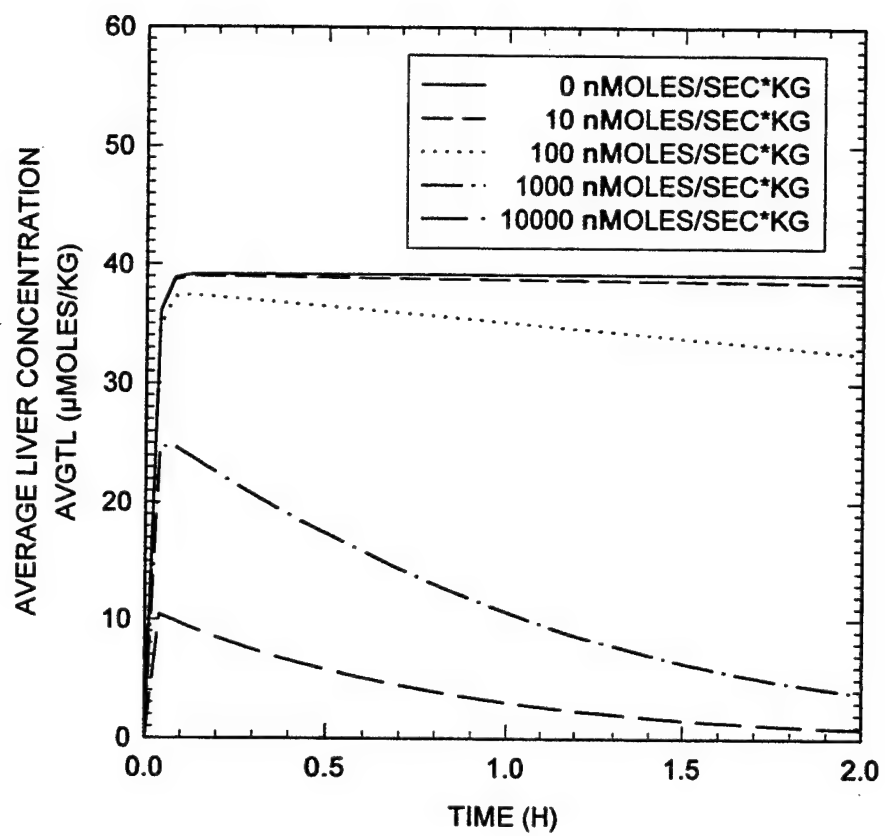
D

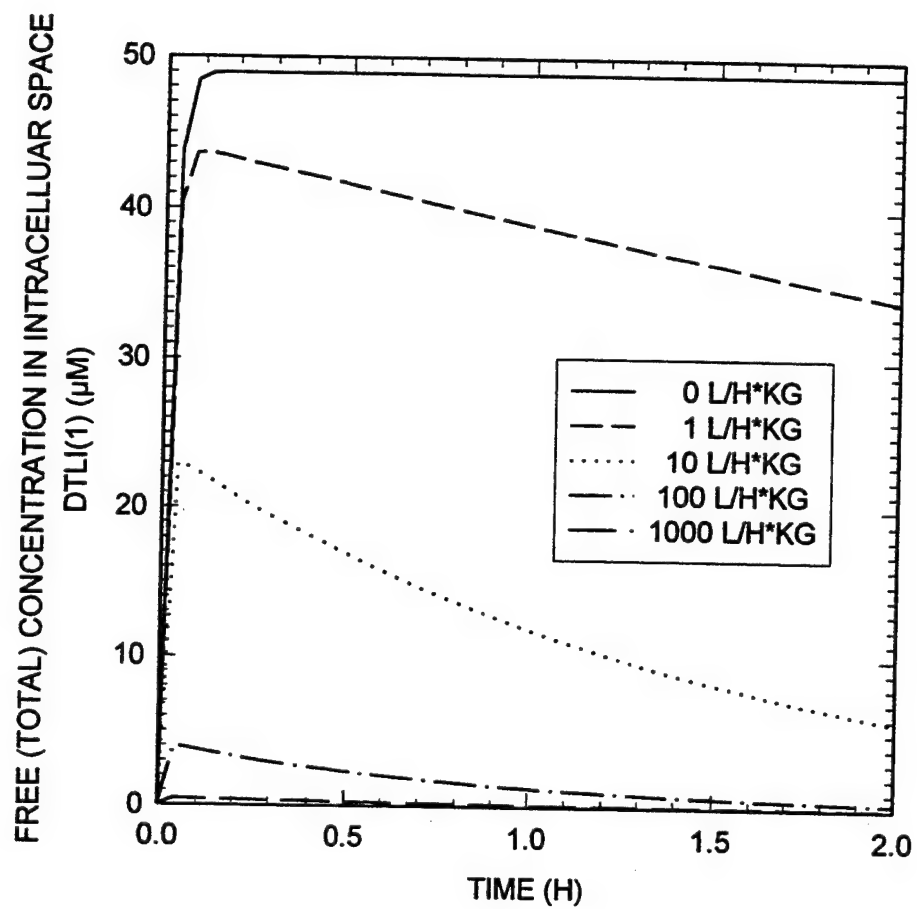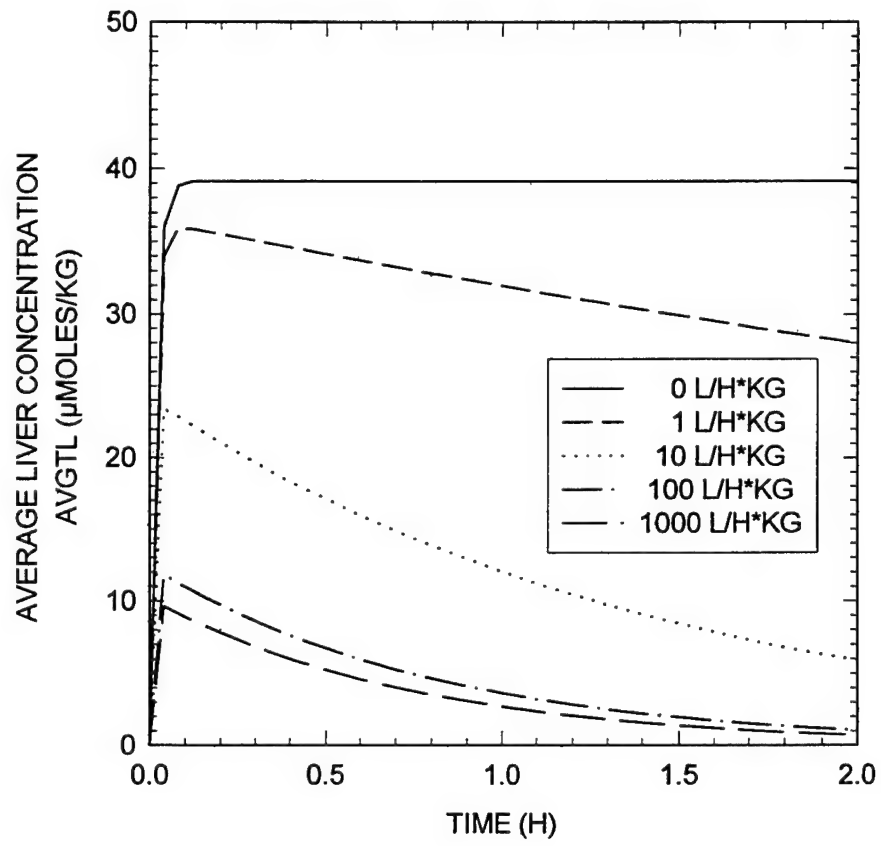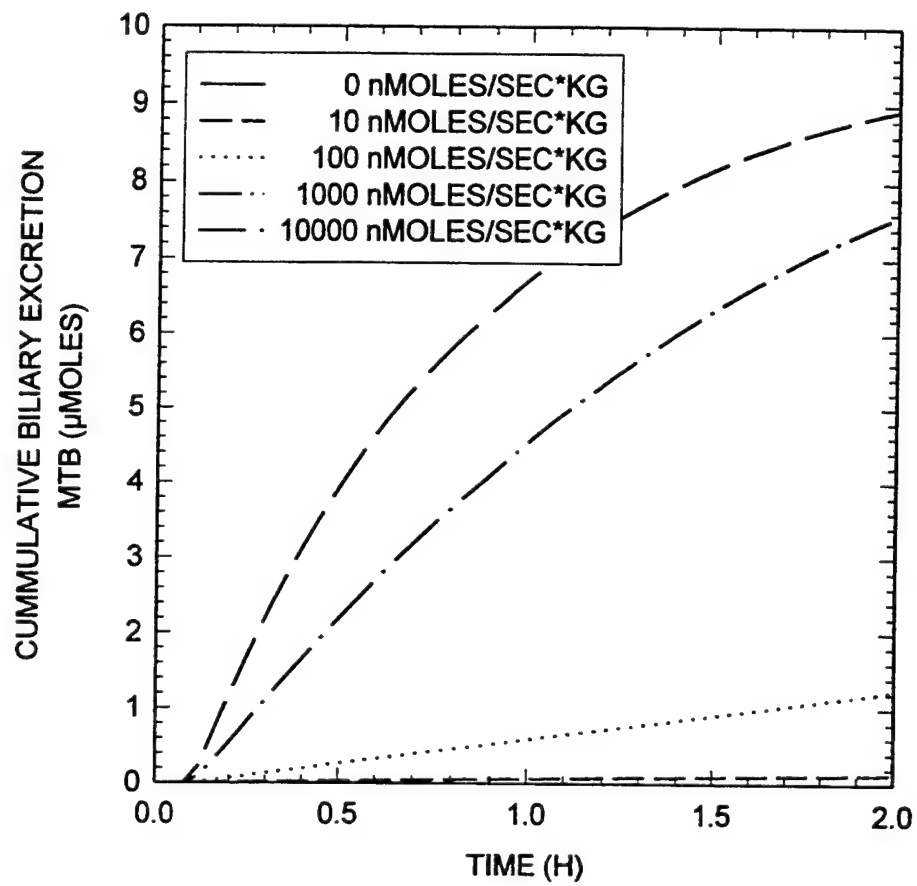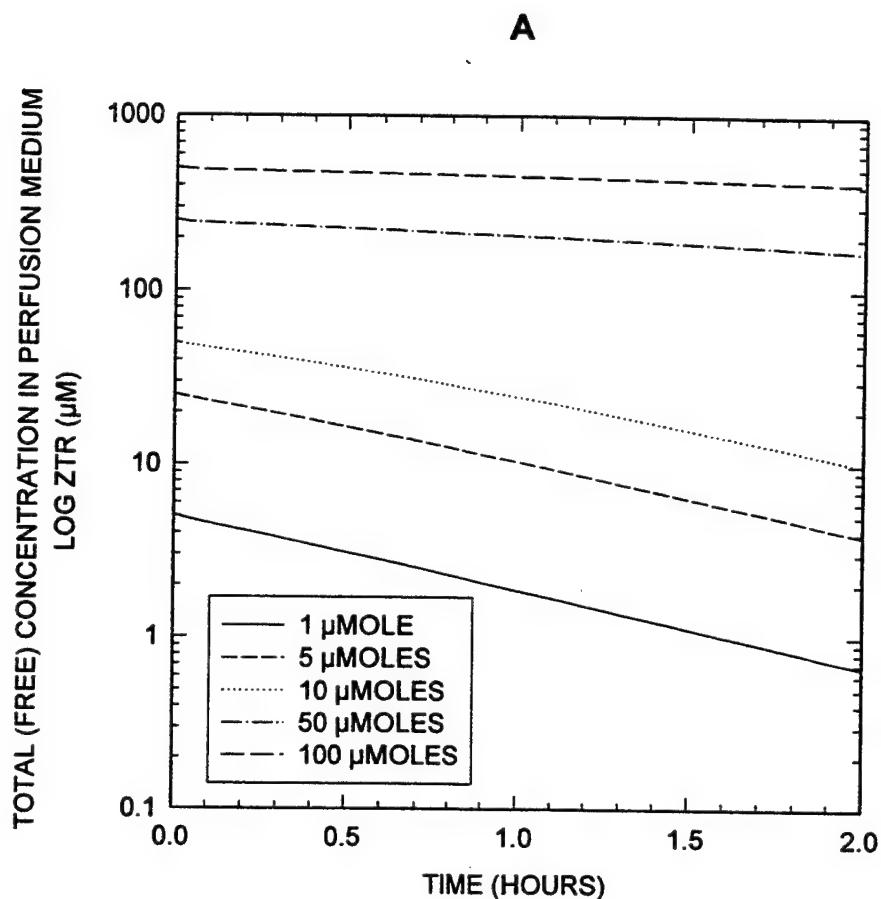**Figure 21: Effect of Dose in the Presence of Saturable Biliary Elimination on the Kinetics of the Parent Chemical T in the Isolated Perfused Rat Liver System.**

(A) Total (free) concentration of the parent chemical in the PM (ZTR), (B) total (free) concentration of the parent chemical in the intracellular water space ((DTLI(1)), and (C) cumulative biliary excretion of the parent chemical (MTB).

B
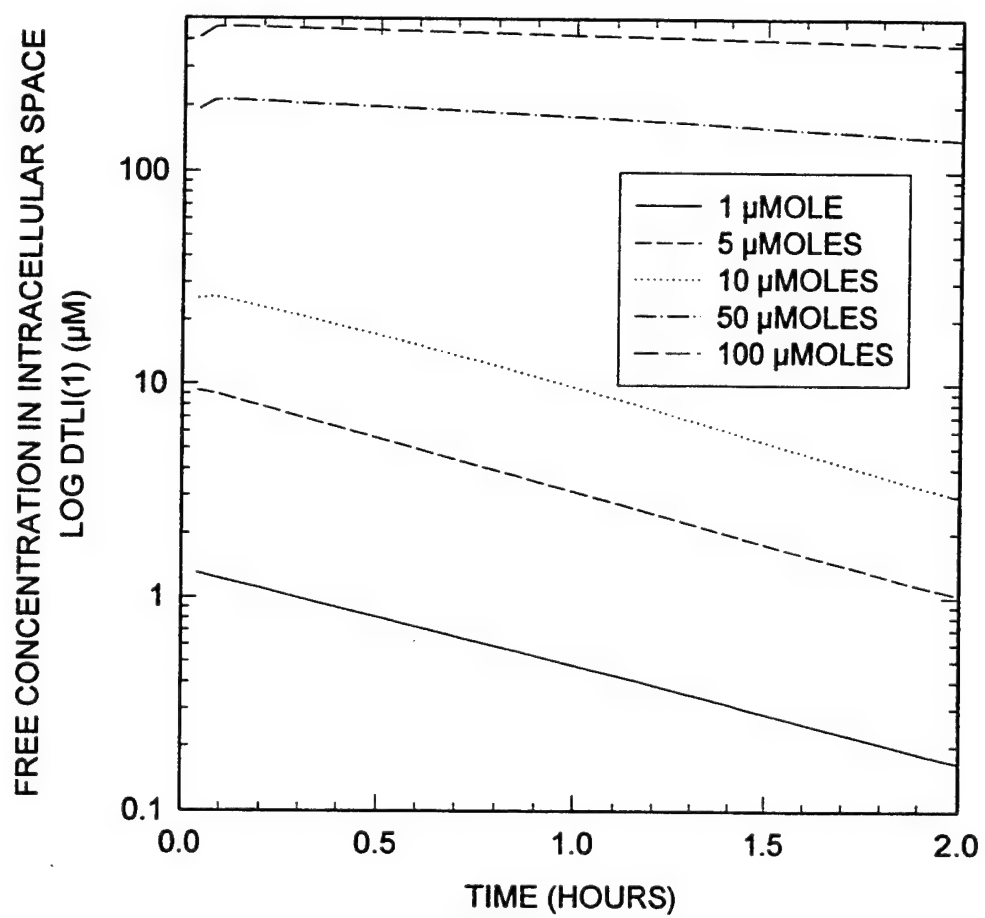
C
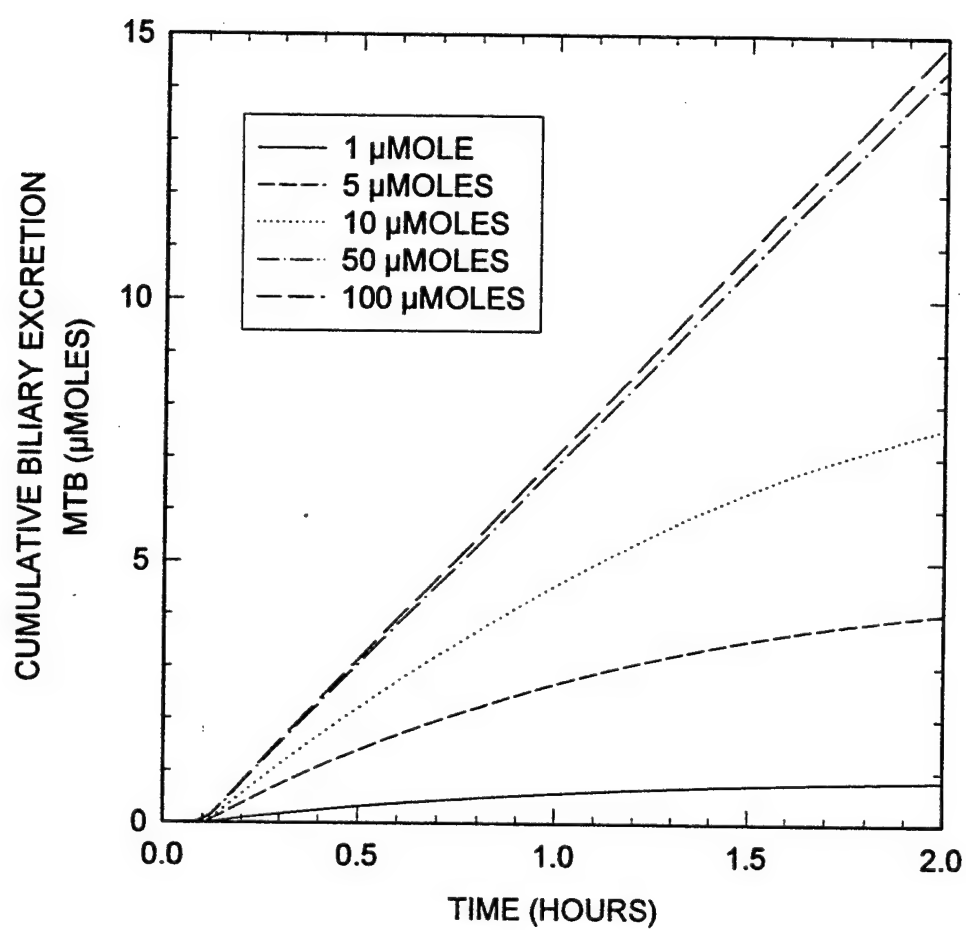
3.0 REFERENCES

Davies, B. and Morris, T. 1993. Physiological parameters in laboratory animals and humans. *Pharmacol. Res.* **10**: 1093-1095.

Goresky, C.A. 1980. Uptake in the liver: The nature of the process. In *Liver and Biliary Tract Physiology,* Vol.21, N.B. Javitt (Ed.). University Park Press, Baltimore, MD. pp. 65-101.

Jain, R.K., Gerlowski, L.E., Weissbrod, J.M., Wang, J. and Pierson, R.N. 1981. Kinetics of uptake, distribution and excretion of zinc in rats. *Ann. Biomed. Eng.* **9**: 347-361.

Wyman, J, Stokes, J.S., Goehring, M., Buring, M. and Moore, T. 1995. Data collection interface for isolated perfused rat liver: Recording oxygen consumption, perfusion pressure and pH. *Toxicol. Methods,* **5**: 1-14.

4.0 APPENDICIES

APPENDIX A: NOMENCLATURE USED IN IPRL MODEL

System Components

> Liver - L
> Reservoir - R

Liver Compartments

> Sinusoids - S
> Intracellular - I

State Variables

> Free Concentrations - X {µM} = DTij(1), DMij(1)
> Bound Concentrations - Y {µM} - DTij(2), DMij(2)
> Total Concentrations - Z {µM}
> Derivatives/Rates - R {µmoles/h}
> Mass - M {µmoles}

Physiological Parameters

> Weight - W {Kg}
> Volume - V {L}
> Area - A {cm^2}
> Fractions - F

Chemical Specific Parameters

Transport parameters

> Diffusion - P {cm/sec}
> Mediated transport - TTijk {µmoles/h*kg}, KTijk {µM}
> Transport coefficients - C {L/h}

Binding Parameters

> Capacity - UBijk {µM}
> Affinity - KBijk (µM}

Metabolism Parameters

Maximum velocity - SVTMAX {µmoles/h∗Kg}
Apparent affinity - KMTij {µM}

APPENDIX B: MODEL PARAMETERS

B.1 CHEMICAL INDEPENDENT PARAMETERS

PARAMETER	VALUE	DESCRIPTION
RESERVOIR		
VR	0.200	Volume of reservoir (L)
QL	2.7	Perfusion rate (L/h)
LIVER		
WL	0.008	Liver weight (Kg)
FVLS	0.128[b]	Fractional sinusoidal volume (L/Kg)
FVLI	0.441[b]	Fractional intracellular volume (L/Kg)
GALSI	1.0	Sinusoidal - intracellular interface area conversion factor

[a] These values for the surface area scaling factors are arbitrary at the present time. Only the permeability * area product is important (see text for discussion).

[b] The sinusoidal volume fraction is the sum of the vascular and interstitial spaces, estimated from the data of Goreski (1980), and the intracellular volume fraction is obtained by subtracting the sinusoidal fractions from the total water fraction in the liver, estimated from the wet/dry ratio determined in this laboratory.

B.2 CHEMICAL SPECIFIC PARAMETERS

B.2.1 Binding parameters in IPRL model.

PARAMETER	VALUE	DESCRIPTION
PERFUSION MEDIUM		
UBTMA	0	Binding capacity of albumin in perfusion medium for parent chemical (μM)
KBTMA	100.0	Dissociation constant for parent chemical binding to albumin (μM)
UBMMA	0	Binding capacity of albumin in perfusion medium for metabolite (μM)
KBMMA	100.0	Dissociation constant for metabolite binding to albumin (μM)
LIVER		
UBTLI	0.0	Chemical binding capacity for parent chemical of HMWM in liver intracellular compartment (μM)
KBTLI	100.0	Dissociation constant for parent chemical binding to HMWM in liver intracellular compartment (μM)
UBMLI	0.0	Chemical binding capacity for metabolite of HMWM in liver intracellular compartment (μM)
KBMLI	100.0	Dissociation constant for metabolite binding to HMWM in liver intracellular compartment (μM)

B.2.2 Transport parameters in IPRL model.

PARAMETER	VALUE	DESCRIPTION
PARENT CHEMICAL TRANSPORT AT SI INTERFACE		
PTTSI = PTTIS	0.1	Diffusional permeability of sinusoidal - intracellular interface for chemical T (cm/sec)
TTTSI	0.0	Maximum chemical transport rate from intracellular to sinusoidal space by mediated transport system (nmoles/sec*cm^2)
TTTIS	0.0	Maximum chemical transport rate from sinusoidal to intracellular space by mediated transport system (nmoles/sec*cm^2)
KTTSI	100	Chemical concentration at half maximum transport rate for intracellular to sinusoidal transport by mediated pathway (µM)
KTTIS	100	Chemical concentration at half maximum transport rate for sinusoidal to intracellular transport by mediated pathway (µM)
METABOLITE TRANSPORT AT SI INTERFACE		
PTMSI = PTMIS	0.1	Diffusional permeability of sinusoidal - intracellular interface for chemical T (cm/sec)
TTMSI	0.0	Maximum chemical transport rate from intracellular to sinusoidal space by mediated transport system (nmoles/sec*cm^2)
TTMIS	0.0	Maximum chemical transport rate from sinusoidal to intracellular space by mediated transport system (nmoles/sec*cm^2)
KTMSI	100	Chemical concentration at half maximum transport rate for intracellular to sinusoidal transport by mediated pathway (µM)
KTMIS	100	Chemical concentration at half maximum transport rate for sinusoidal to intracellular transport by mediated pathway (µM)

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B.2.3 Metabolism parameters for IPRL model.

PARAMETER	VALUE	DESCRIPTION
SVTMAX	0.0	Maximum rate of metabolism (μmoles/h*Kg)
KMT	1.0	K_M for enzymatic metabolism of T (μM)

B.2.4 Biliary excretion parameters for IPRL model.

PARAMETER	VALUE	DESCRIPTION
PARENT CHEMICAL		
STTB1	0.0	Rate constant for linear transport of T (L/H*Kg)
STTB2	0.0	Maximum rate for saturable transport of T (μmoles/h*Kg)
KTTB	100	K_T for biliary excretion of T (μM)
METABOLITE		
STMB1	0.0	Rate constant for linear transport of M (L/h*Kg)
STMB2	0.0	Maximum rate for saturable transport of M (μmoles/h*Kg)
KTMB	100	K_T for biliary excretion of M (μM)
DELAY		
BDELAY	0.1	Time delay for collection of bile (H)

B.3 Simulation control parameters for IPRL model.

Control parameters for solution of nonlinear binding equations

PARAMETER	VALUE	DESCRIPTION
TOL	100.0	Error criteria factor
ITER	100	Maximum number of iterations attempted

Chemical dosing

PARAMETER	VALUE	DESCRIPTION
MTDOSE	0.0	Amount of parent chemical added to the reservoir (μmoles)

Simulation parameters

PARAMETER	VALUE	DESCRIPTION
TSTOP	2.0	Length of simulation (h)
POINTS	1000	Total number of data transfers to storage
IALG	2	Integration algorithm (2 = Gear variable step algorithm)

APPENDIX C: MODEL CODE

PROGRAM IPRLR40

! FILE NAME =IPRLR40.CSL STORED IN L:\MODELS\IPRLMOD
! JOHN M. FRAZIER - PROGRAM DEVELOPMENT INITIATED 15 APR 96
! DATE OF LAST PROGRAM ALTERATION 28 APR 97

! 28 APR 97 - THIS IS THE COMPLETE MODEL FOR THE RECIRCULATION IPRL SYSTEM
! THE FOLLOWING PROPERTIES ARE INCLUDED IN THE MODEL:

! (1) MODEL FOR RECIRCULATING SYSTEM
! (2) CONSIDER LIVER AS TWO WATER COMPARTMENTS - SINUSOIDS AND INTRACELLULAR
!   SPACES
! (3) TIME BEGINS AT -1 H
! (4) CORRECTION MADE FOR VOLUME CHANGES DURING SAMPLING
! (5) TRANSPORT EQUATIONS CORRECTED FOR NORMAL UNITS OF P
! (6) TIME DELAY FOR BILIARY EXCRETION INCLUDED
! (7) INCLUDES DESCRIPTION OF METABOLITE
! (8) THE LOSS OF CHEMICAL FROM THE LIVER AS A RESULT OF LOSS OF LIVER
!   PLASMA MEMBRANE INTEGRITY

!===

INITIAL

! PHYSIOLOGICAL PARAMETERS FOR LIVER

CONSTANT	QL	= 2.7	! PERFUSION RATE (L/H)
CONSTANT	WL	= 0.008	! WEIGHT OF LIVER (L)
CONSTANT	FVLS	= 0.217	! VOLUME FRACTION OF...
			! LIVER SINUSOIDS
CONSTANT	FVLI	= 0.583	! VOLUME FRACTION OF...
			! LIVER INTRACELLULAR SPACE
CONSTANT	GALI	= 1.0	! SINUSOIDAL-INTRACELLULAR...
			! SURFACE AREA SCALE FACTOR (CM2/KG)
CONSTANT	VR0	= 0.200	! INITIAL VOLUME OF PERFUSION MEDIUM (L)
VR		= VR0	! INITIALIZE VOLUME OF PERFUSION MEDIUM (L)

! ***************** TRANSPORT PARAMETERS *********************************

! T TRANSPORT AT SINUSOIDAL-INTRACELLULAR INTERFACE

CONSTANT	PTTSI	= 10.0	! PERMEABILITY OF T I TO S (CM/SEC)
CONSTANT	PTTIS	= 10.0	! PERMEABILITY OF T S TO I (CM/SEC)
CONSTANT	TTTSI	= 0.0	! MEDIATED TRANSPORT OF T I TO S -
			RATE CONSTANT (μMOLES/H*CM^2)
CONSTANT	TTTIS	= 1.0	! MEDIATED TRANSPORT OF T S TO I -
			RATE CONSTANT (μMOLES/H*CM^2)
CONSTANT	KTTSI	= 0.1	! MEDIATED TRANSPORT OF T I TO S -
			AFFINITY (μM)
CONSTANT	KTTIS	= 1.0	! MEDIATED TRANSPORT OF T I TO S -
			AFFINITY (μM)

! M TRANSPORT AT SINUSOIDAL-INTRACELLULAR INTERFACE

```
CONSTANT    PTMSI = 10.0          ! PERMEABILITY OF M I TO S (CM/SEC)
CONSTANT    PTMIS = 10.0          ! PERMEABILITY OF M S TO I (CM/SEC)
CONSTANT    TTMSI = 0.0           ! MEDIATED TRANSPORT OF M I TO S -
                                    RATE CONSTANT (μMOLES/H*CM²)
CONSTANT    TTMIS = 1.0           ! MEDIATED TRANSPORT OF M S TO I -
                                    RATE CONSTANT (μMOLES/H*CM²)
CONSTANT    KTMSI = 0.1           ! MEDIATED TRANSPORT OF M I TO S -
                                    AFFINITY (μM)
CONSTANT    KTMIS = 1.0           ! MEDIATED TRANSPORT OF M I TO S -
                                    AFFINITY (μM)
```

! BILIARY EXCRETION OF T

```
CONSTANT    BDELAY = 0.1          ! TIME DELAY FOR BILE COLLECTION (H)
CONSTANT    SPTTB = 0.0           ! LINEAR EXCRETION OF T (L/H*KG)
CONSTANT    STTTB = 0.0           ! MEDIATED EXCRETION OF T - RATE CONSTANT
                                    (μMOLES/H*KG)
CONSTANT    KTTB  = 1.0           ! MEDIATED EXCRETION OF T - AFFINITY
                                    (μMOLES/H*KG)
```

! BILIARY EXCRETION OF M

```
CONSTANT    SPTMB = 0.0           ! LINEAR EXCRETION OF M (L/H*KG)
CONSTANT    STTMB = 0.0           ! MEDIATED EXCRETION OF M - RATE CONSTANT
                                    (μMOLES/H*KG)
CONSTANT    KTMB  = 1.0           ! MEDIATED EXCRETION OF M - AFFINITY
                                    (μMOLES/H*KG)
```

! ******************** T METABOLISM **

```
CONSTANT    SVTMAX = 0.0          ! MAXIMUM RATE OF METABOLISM
                                    PER KG LIVER TISSUE (μMOLES/H/KG)
CONSTANT    KMTLI = 1.0           ! KM FOR METABOLISM (μM)
RTMAX       = SVTMAX*WL           ! METABOLISM RATE CONSTANT (μMOLES/H)
```

! ******************** T BINDING PARAMETERS ******************************

```
CONSTANT    UBTP  = 0.0           ! BINDING CAPACITY FOR T IN PLASMA (μM)
CONSTANT    KBTP  = 100.0         ! BINDING AFFINITY FOR T IN PLASMA (μM)
CONSTANT    UBMP  = 0.0           ! BINDING CAPACITY FOR M IN PLASMA (μM)
CONSTANT    KBMP  = 100.0         ! BINDING AFFINITY FOR M IN PLASMA (μM)
CONSTANT    UBTLI = 0.0           ! BINDING CAPACITY FOR T IN LI (μM)
CONSTANT    KBTLI = 100.0         ! BINDING AFFINITY FOR T IN LI (μM)
CONSTANT    UBMLI = 0.0           ! BINDING CAPACITY FOR M IN LI (μM)
CONSTANT    KBMLI = 100.0         ! BINDING AFFINITY FOR M IN LI (μM)
```

!******************** CALCULATED PARAMETERS ******************************

```
VLS         = FVLS*WL            ! VOLUME OF SINUSOIDAL SPACE(L)
VLI         = FVLI*WL            ! VOLUME OF INTRACELLULAR SPACE (L)
ALI         = GALI*WL            ! SURFACE AREA OF SINUSOIDAL - INTRACELLULAR
                                   INTERFACE (CM2)
```

!******************** INITIAL CONDITIONS *********************************

! INITIAL CONDITIONS FOR T IN LIVER COMPARTMENTS

```
CONSTANT    ZTLS0  = 0.0
MTLS0              = ZTLS0*VLS
CONSTANT    ZTLI0  = 0.0
MTLI0              = ZTLI0*VLI
```

! INITIAL CONDITIONS FOR T IN BILE

```
CONSTANT    MTB0   = 0.0
```

! INITIAL CONDITIONS FOR T IN RESERVOIR

```
CONSTANT    ZTR0   = 0.0
MTR0               = ZTR0*VR
```

! INITIAL CONDITIONS FOR M IN LIVER COMPARTMENTS

```
CONSTANT    ZMLS0  = 0.0
MMLS0              = ZMLS0*VLS
CONSTANT    ZMLI0  = 0.0
MMLI0              = ZMLI0*VLI
```

! INITIAL CONDITIONS FOR M IN BILE

```
CONSTANT    MMB0   = 0.0
```

! INITIAL CONDITIONS FOR M IN RESERVOIR

```
CONSTANT    ZMR0   = 0.0
MMR0               = ZMR0*VR
```

! INITIALIZE CUMMULATIVE LOSS OF T AND M BY SAMPLING

```
MTS    = 0.0
MMS    = 0.0
```

!******************* INITIALIZE STATE VARIABLES *****************************

! STATE VARIABLES FOR T

```
ARRAY DTLS(2),DTLI(2),DTR(2)
```

! INITIALIZE FREE AND BOUND T IN SINUSOIDS

```
PROCEDURAL(DTLS=MTLS0,UBTP,KBTP,VLS)

     CALL DIST11(DTLS,MTLS0,UBTP,KBTP,VLS)

END

ZTLS   = MTLS0/VLS
```

```
! INITIALIZE FREE AND BOUND T IN LIVER INTRACELLULAR SPACE

        PROCEDURAL(DTLI=MTLI0,UBTLI,KBTLI,VLI)

                CALL DIST11(DTLI,MTLI0,UBTLI,KBTLI,VLI)

        END

! INITIALIZE FREE AND BOUND T IN RESERVOIR

        PROCEDURAL(DTR=MTR0,UBTP,KBTP,VR)

                CALL DIST11(DTR,MTR0,UBTP,KBTP,VR)

        END

        ZTR     = MTR0/VR

! STATE VARIABLES FOR M

        ARRAY DMLS(2),DMLI(2),DMR(2)

! INITIALIZE FREE AND BOUND M IN SINUSOIDAL SPACE

        PROCEDURAL(DMLS=MMLS0,UBMP,KBMP,VLS)

                CALL DIST11(DMLS,MMLS0,UBMP,KBMP,VLS)

        END

        ZMLS    = MMLS0/VLS

! INITIALIZE FREE AND BOUND M IN LIVER INTRACELLULAR SPACE

        PROCEDURAL(DMLI=MMLI0,UBMLI,KBMLI,VLI)

                CALL DIST11(DMLI,MMLI0,UBMLI,KBMLI,VLI)

        END

! INITIALIZE FREE AND BOUND M IN RESERVOIR

        PROCEDURAL(DMR=MMR0,UBMP,KBMP,VR)

                CALL DIST11(DMR,MMR0,UBMP,KBMP,VR)

        END

        ZMR     = MMR0/VR

!***************************** DOSING *****************************************

        CONSTANT    MTDOSE      = 0.0
```

```
!********************** SIMULATION PARAMETERS *******************************

      ALGORITHM    IALG   = 2            ! GEAR NUMERICAL ...
                                         ! INTEGRATION ALGORITHM
      CONSTANT     ALFLAG = 0.0          ! CONTROL FOR LEAKAGE

!************************* RUN PARAMETERS ************************************

      VARIABLE     TIME, TIMEIC  = - 1.0
      CONSTANT     TSTOP         = 1           ! LENGTH OF SIMULATION (H)
      CONSTANT     POINTS        = 1000        ! NUMBER OF DATA TRANSFERS
      CINT         = TSTOP/POINTS              ! INTERVAL FOR SAVING DATA
      CONSTANT     DOSET         =0.0

!********************** SAMPLING CONTROL ************************************

      ARRAY TS(10),SV(10),TS1(8),TS2(8),LDH(8)
      CONSTANT     TS(1)=-0.5,TS(2)=0.0,TS(3)=0.0333,TS(4)=0.0833
      CONSTANT     TS(5)=0.1667,TS(6)=0.333,TS(7)=0.5,TS(8)=1.0
      CONSTANT     TS(9)=1.5,TS(10)=2.0
      CONSTANT     SV(1)=0.001,SV(2)=0.001,SV(3)=0.001,SV(4)=0.001
      CONSTANT     SV(5)=0.001,SV(6)=0.001,SV(7)=0.0025,SV(8)=0.0025
      CONSTANT     SV(9)=0.0025,SV(10)=0.0025
      CONSTANT     TS1(1)=-0.5,TS1(2)=0.0,TS1(3)=0.5,TS1(4)=1.0
      CONSTANT     TS1(5)=1.5,TS1(6)=2.0,TS1(7)=5.0,TS1(8)=6.0
      CONSTANT     TS2(1)=-0.5,TS2(2)=0.0,TS2(3)=0.5,TS2(4)=1.0
      CONSTANT     TS2(5)=1.5,TS2(6)=2.0,TS2(7)=5.0,TS2(8)=6.0
      CONSTANT     LDH(1)=0.0,LDH(2)=0.0,LDH(3)=0.0,LDH(4)=0.0
      CONSTANT     LDH(5)=0.0,LDH(6)=0.0,LDH(7)=0.0,LDH(8)=0.0
      INTEGER      ISAMP,ISAMP1
      ISAMP        = 1
      ISAMP1       = 1
      SAMPV        = SV(1)
      RLDH   = (LDH(ISAMP1+1) - LDH(ISAMP1))/(TS1(ISAMP1+1) - TS1(ISAMP1))


END                                          ! END OF INITIAL SECTION

!=======================================================================

DYNAMIC

!=======================================================================

DERIVATIVE

! **************** CONTROL FOR SAMPLING VOLUME CORRECTION ******************

      SCHEDULE     DOSE .XP. TIME-DOSET

      SCHEDULE     SAMPLE .XP. TIME-TS(1)
      SCHEDULE     SAMPLE .XP. TIME-TS(2)
      SCHEDULE     SAMPLE .XP. TIME-TS(3)
      SCHEDULE     SAMPLE .XP. TIME-TS(4)
```

```
SCHEDULE      SAMPLE .XP. TIME-TS(5)
SCHEDULE      SAMPLE .XP. TIME-TS(6)
SCHEDULE      SAMPLE .XP. TIME-TS(7)
SCHEDULE      SAMPLE .XP. TIME-TS(8)
SCHEDULE      SAMPLE .XP. TIME-TS(9)
SCHEDULE      SAMPLE .XP. TIME-TS(10)


SCHEDULE      LEAK .XP. TIME-TS1(1)
SCHEDULE      LEAK .XP. TIME-TS1(2)
SCHEDULE      LEAK .XP. TIME-TS1(3)
SCHEDULE      LEAK .XP. TIME-TS1(4)
SCHEDULE      LEAK .XP. TIME-TS1(5)
SCHEDULE      LEAK .XP. TIME-TS1(6)
SCHEDULE      LEAK .XP. TIME-TS1(7)
SCHEDULE      LEAK .XP. TIME-TS1(8)


SCHEDULE      BILE .XP. TIME-TS2(1)
SCHEDULE      BILE .XP. TIME-TS2(2)
SCHEDULE      BILE .XP. TIME-TS2(3)
SCHEDULE      BILE .XP. TIME-TS2(4)
SCHEDULE      BILE .XP. TIME-TS2(5)
SCHEDULE      BILE .XP. TIME-TS2(6)
SCHEDULE      BILE .XP. TIME-TS2(7)
SCHEDULE      BILE .XP. TIME-TS2(8)
```

! *********************** DE FOR LIVER (L) **********************************

! TRANSPORT PARAMETERS FOR T AND M IN LIVER

```
CTLSI  = TRANS(PTTSI,TTTSI,KTTSI,DTLI(1),ALI)
CTLIS  = TRANS(PTTIS,TTTIS,KTTIS,DTLS(1),ALI)
CTLB   = TRANS(SPTTB,STTTB,KTTB,DTLI(1),WL)
CMLSI  = TRANS(PTMSI,TTMSI,KTMSI,DMLI(1),ALI)
CMLIS  = TRANS(PTMIS,TTMIS,KTMIS,DMLS(1),ALI)
CMLB   = TRANS(SPTMB,STTMB,KTMB,DMLI(1),WL)
```

! TOTAL T IN L SINUSOIDAL SPACE (LS)

    ! RATE OF LOSS OF T FROM LIVER DUE TO NECROSIS

```
RLT    = ALFLAG*RLDH*WL*FVLI*ZTLI

RMTLS = QL*ZTR + CTLSI*DTLI(1) - CTLIS*DTLS(1) &
                 + RLT - QL*ZTLS

MTLS   = INTEG(RMTLS, MTLS0)
```

! DISTRIBUTION OF T BETWEEN X AND Y IN LS

```
PROCEDURAL(DTLS=MTLS,UBTP,KBTP,VLS)
```

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```
                    CALL DIST11(DTLS,MTLS,UBTP,KBTP,VLS)

        END

        ZTLS    = MTLS/VLS

! TOTAL T IN INTRACELLULAR COMPARTMENT OF L (LI)

        RMML  = RTMAX*DTLI(1)/(DTLI(1) + KMTLI)

        RMTB  = CTLB*DTLI(1)

        RMTLI = CTLIS*DTLS(1) - CTLSI*DTLI(1) - RLT- RMML - RMTB

        MTLI   = INTEG(RMTLI, MTLI0)

! DISTRIBUTION OF T BETWEEN X AND Y IN LI

        PROCEDURAL(DTLI=MTLI,UBTLI,KBTLI,VLI)

                    CALL DIST11(DTLI,MTLI,UBTLI,KBTLI,VLI)

        END

        ZTLI    = MTLI/VLI

! TOTAL M IN L SINUSOIDAL SPACE (LS)

        RLM     = ALFLAG*RLDH*WL*FVLI*ZMLI        ! RATE OF LOSS OF M FROM ...
                                                  ! LIVER DUE TO NECROSIS

        RMMLS           = QL*ZMR + CMLSI*DMLI(1) - CMLIS*DMLS(1) &
                        + RLM - QL*ZMLS

        MMLS  = INTEG(RMMLS,MMLS0)

! DISTRIBUTION OF M BETWEEN X AND Y IN LS

        PROCEDURAL(DMLS=MMLS,UBMP,KBMP,VLS)

                    CALL DIST11(DMLS,MMLS,UBMP,KBMP,VLS)

        END

        ZMLS   = MMLS/VLS

! TOTAL M IN INTRACELLULAR COMPARTMENT OF L (LI)

        RMMB  = CMLB*DMLI(1)

        RMMLI = CMLIS*DMLS(1) - CMLSI*DMLI(1) - RLM + RMML - RMMB

        MMLI   = INTEG(RMMLI, MMLI0)
```

! DISTRIBUTION OF M BETWEEN X AND Y IN LI

      PROCEDURAL(DMLI=MMLI,UBMLI,KBMLI,VLI)

          CALL DIST11(DMLI,MMLI,UBMLI,KBMLI,VLI)

      END

      ZMLI   = MMLI/VLI

! CUMMULATIVE EXCRETION OF T IN COLLECTED BILE

      RMTBD= DELAY(RMTB,0.0,BDELAY,200,0.001)

      MTB   = INTEG(RMTBD,MTB0)

! CUMMULATIVE EXCRETION OF M IN COLLECTED BILE

      RMMBD   = DELAY(RMMB,0.0,BDELAY,200,0.001)

      MMB = INTEG(RMMBD,MMB0)

! TOTAL T IN L

      MTL   = MTLS + MTLI

! TOTAL M IN L

      MML   = MMLS + MMLI

! AVERAGE CONCENTRATION OF T IN LIVER

      AVGTL = MTL/WL   ! SINUSOIDAL SPACE INCLUDED

      AVGTLC   = MTLI/WL   ! WASHED LIVER

! AVERAGE CONCENTRATION OF M IN LIVER

      AVGML   = MML/WL   ! SINUSOIDAL SPACE INCLUDED

      AVGMLC   = MMLI/WL   ! WASHED LIVER

! AVERAGE CONCENTRATION OF TOTAL (T + M) IN LIVER (TOTAL RADIOACTIVITY)

      AVGL = AVGTL + AVGML   ! SINUSOIDAL SPACE INCLUDED

      AVGLC = AVGTLC + AVGMLC   ! WASHED LIVER

!*********************** DE FOR RESERVOIR *********************************

! TOTAL T IN RESERVOIR (R)

      RMTR = QL*(ZTLS - ZTR)

```
        MTR    = INTEG(RMTR,MTR0)

! DISTRIBUTION OF T BETWEEN X AND Y IN R

        PROCEDURAL(DTR=MTR,UBTP,KBTP,VR)

                CALL DIST11(DTR,MTR,UBTP,KBTP,VR)

        END

        ZTR    = MTR/VR

! TOTAL M IN RESERVOIR (R)

        RMMR  = QL*(ZMLS - ZMR)

        MMR    = INTEG(RMMR,MMR0)

! DISTRIBUTION OF M BETWEEN X AND Y IN R

        PROCEDURAL(DMR=MMR,UBMP,KBMP,VR)

                CALL DIST11(DMR,MMR,UBMP,KBMP,VR)

        END

        ZMR    = MMR/VR

! CONCENTRATION OF TOTAL (T + M) IN RESERVOIR (TOTAL RADIOACTIVITY)

        ZTOTR = ZTR + ZMR

! RECOVERY

        MTOT  = MTLS + MTLI + MTR + MTB + MMLS + MMLI+ MMR + MMB + MTS + MMS

!***************** TERMINATION CONDITION ***********************************

        TERMT(TIME .GE. TSTOP)              ! STOP SIMULATION WHEN...
                                           ! T>=TSTOP

END                                        ! END OF DERIVATIVE SECTION

!======================================================================

DISCRETE      DOSE

        MTR    = MTR + MTDOSE

END

DISCRETE      SAMPLE

        VR     = VR - SAMPV
```

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```
      MTR    = MTR - ZTR*SAMPV
      MTS    = MTS + ZTR*SAMPV
      MMR    = MMR - ZMR*SAMPV
      MMS    = MMS + ZMR*SAMPV
      ISAMP  = ISAMP + 1
      SAMPV  = SV(ISAMP)

    END                                    ! END OF DISCRETE SECTION SAMPLE

  DISCRETE     LEAK

      RLDH   = (LDH(ISAMP1+1) - LDH(ISAMP1))/(TS1(ISAMP1+1) - TS1(ISAMP1))
      ISAMP1 = ISAMP1 + 1

    END                                    ! END OF DISCRETE SECTION LEAK

  DISCRETE     BILE

      TCUMB  = MTB
      MCUMB          = MMB

    END                                    ! END OF DISCRETE SECTION BILE

!===============================================================================

  END                                      ! END OF DYNAMIC SECTION

!===============================================================================
  END                                      ! END OF ACSL PROGRAM
      SUBROUTINE DIST11(D,M,U,K,V)
*
      REAL*8        D(2),M,U,K,V
      REAL*8        MT,UT,ZT,VT
      LOGICAL       DFLAG1,DFLAG2,DFLAG3
*
* IF M IS NEGATIVE THEN DFLAG1 IS SET .TRUE. *
      DFLAG1        = .FALSE.
* IF U IS NEGATIVE THEN DFLAG2 IS SET .TRUE. *
      DFLAG2        = .FALSE.
* IF V IS NEGATIVE THEN DFLAG3 IS SET .TRUE. *
      DFLAG3        = .FALSE.
*
      IF (M .LT. 0.0) THEN
         DFLAG1     = .TRUE.
         MT         = - M
      ELSE
         MT         = M
      END IF
*
      IF (U .LT. 0.0) THEN
         DFLAG2     = .TRUE.
         UT         = -U
      ELSE
         UT         = U
```

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```
      END IF
*
      IF (V .LT. 0.0) THEN
         DFLAG3    = .TRUE.
         VT        = - V
      ELSE
         VT        = V
      END IF
*
      ZT           = MT/VT
*
      IF ((U .EQ. 0.0)) THEN
         D(1)      = ZT
         D(2)      = 0.0
      ELSE
         D(1)      = (SQRT((K + UT - ZT)**2
     1 + 4*K*ZT) - (K + UT - ZT))/2.0
         D(2)      = ZT - D(1)
      END IF
*
      IF (DFLAG1) THEN
         D(1)      = - D(1)
         D(2)      = - D(2)
      END IF
*
120   CONTINUE
      RETURN
      END
******************************************************************************
      REAL*8 FUNCTION TRANS(P,T,K1,X1,A)
*
      REAL*8        P,T,K1,X1,A
*
      TRANS    = 3.6*P*A + (T*A)/(X1 + K1)
      RETURN
      END
```